extract was very much lower than that from oxythiamine and pyrithiamine, suggesting that the enzyme activity required for amination of 2-methyl-4-hydroxy-5-hydroxymethylpyrimidine to 2-methyl-4-amino-5-hydroxymethylpyrimidine is extremely weak in the cell-free extract of *S. cerevisiae*. From these results it was concluded that *S. cerevisiae* contains thiaminase II in addition to a thiamine synthesizing enzyme system to produce thiamine from pyrimidine and thiazole moieties of thiamine. Ozawa et al.[11] previously reported the presence of thiaminase activity in some yeast-like fungi. The precise enzymatic mechanism of thiamine synthesis from pyrithiamine and oxythiamine in *S. cerevisiae* remains to be established.

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0014-4754/84/060582-02$1.50 + 0.20/0

Volatile metabolites of aspergilli in relation to spore germination of some keratinophilic fungi[1]

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Summary. The effect of volatile metabolites produced by 8 Aspergilli (i.e., *Aspergillus candidus, A. chevalieri, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochraceous* and *A. tamarii*) on spore germination were tested against *Auxarthron conjugatum, Chrysosporium pannicola, Keratinomyces ajelloi* and *Microsporum gypseum*. The volatile metabolites inhibited the spore germination of all the test fungi.

During the microbial degradation of organic waste a number of volatile compounds are produced. These volatiles are either produced by microorganisms or by decaying organic matter. Soil-inhabiting keratinophilic fungi in all habitats live in a atmosphere of volatile organic compounds, which are known to play an important role in soil fungistasis[2–6]. To add to the knowledge available on this topic, the production of volatiles by Aspergilli was investigated and their inhibitory/stimulatory effect against keratinophilic fungi was evaluated.

The volatile metabolites produced by 8 Aspergilli (table) were tested against *Auxarthron conjugatum, Chrysosporium pannicola, Keratinomyces ajelloi* and *Microsporum gypseum* isolated during a survey of soil-inhabiting keratinophilic fungi. The effect of volatiles on spore germination was evaluated by 2 assay techniques. *Sterile cellophane agar diffusion (SCAD) method*[4]. In this method agar discs (6 mm diameter and 2–3 mm thick) made from 2% Difco purified agar were placed on sterile cellophane (2 × 2 inches) in contact with 35 g of sterilized soil in the petri dishes. The petri dishes were inoculated with individual *Aspergillus* species. Inoculations were made 10 days before adding the cellophane paper and discs. The agar discs were preactivated in this manner for 24 h at 28 °C. They were transferred to a sterile glass slide in a glass tube kept in a moistened petri dish and then inoculated with spore suspension of a 10-day-old test organism grown on Sabouraud's, dextrose agar medium. After 24 h incubation at 28 °C, the agar discs were examined for spore germination of the test fungi. Approximately 200 conidia were observed in each count.

Soil emanation agar (SEA) method[4]. In this method a sterile glass slide was attached in the center of the inside of a petri dish cover with masking tape, and the agar discs were placed on its free surface. The cover of the petri dish with the slide and agar discs was replaced on the bottom plate containing a pure culture of 10-day-old Aspergilli. The agar discs were thus exposed to the volatile substances emanating from the culture. Preactivation and the remaining procedures were the same as described in the previous method.

The result presented in the table showed that the volatile metabolites inhibit the spore germination of all the test fungi. The volatiles produced by *A. chevalieri* and *A. ochraceous* could cause 100% inhibition in spore germination of *K. ajelloi*, while in the case of *M. gypseum* the volatiles produced by *A. cheva-*

Sporostatic effect of volatiles produced by different species of *Aspergillus*

S. No.	*Aspergillus* spp.	% inhibition in spore germination of keratinophilic fungi							
		A. conjugatum		*C. pannicola*		*K. ajelloi*		*M. gypseum*	
		A	B	A	B	A	B	A	B
1	*Aspergillus candidus*	44	53	52	59	68	73	48	40
2	*A. chevalieri*	59	41	64	61	100	100	100	100
3	*A. flavus*	52	49	63	57	49	53	74	83
4	*A. fumigatus*	33	19	76	71	25	07	70	68
5	*A. nidulans*	70	67	90	93	78	75	54	58
6	*A. niger*	70	66	72	76	59	63	77	72
7	*A. ochraceous*	74	88	62	55	100	100	95	93
8	*A. tamarii*	58	54	45	43	68	64	60	56

A, Sterile cellophane agar diffusion method; B, soil emanation agar method. Each figure is an average of 3 determinations.

lieri could cause the same type of effect. The volatile emanations from *A. ochraceous* were found to be highly toxic for the spore germination of *M. gypseum* showing 95% inhibition when tested by the SCAD method, and 93% inhibition was recorded in the SEA method.

Among the fungi a range of sensitivity exists[5-7]. The fungal populations used for the study behave differently in response to the volatile substances produced by the soil fungi, as they differ in their membrane structure, gaseous diffusion through the membrane and the nature of the volatile produced. In different situations, different Aspergilli may dominate the soil fungal flora. This may play an important role in determining the prevalence of keratinophilic fungi and related dermatophytes in the soil, since, as was shown here for *A. conjugatum*, *C. pannicola*, *K. ajelloi* and *M. gypseum*, not all species react in the same way to the sporostatic factors produced.

1 Acknowledgment. The authors thank Professor G.P. Mishra for the use of the facilities and S.K.D. acknowledges the fellowship award by I.C.M.R. New Delhi.

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0014-4754/84/060583-02$1.50 + 0.20/0

Anesthetization of Planorbidae (Mollusca): methodology and pharmacology[1]

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Summary. A slow drip application of sodium pentobarbital was used to anesthetize *Helisoma duryi* for surgery. The use of a 0.01 M Trizma buffer in the anesthetic medium expedites recovery and appeared to reduce swelling of the pedal sinus. These observations are attributed to the restriction of ΔpH favoring the entry of anesthetic into tissues and reducing osmotic stress experienced by the animal.

Anesthesia of gastropods has been employed for relaxation prior to fixation[2-11], injection and/or hemolymph sampling[12,13], and surgery[7,14-16]. Variation in the response to anesthetics has been reported not only between genera but also between species of the same genus. Of the numerous agents employed to date, sodium pentobarbital used either alone or in conjunction with other relaxing agents, produces the most reliable and consistent anesthesia[13,15,16]. Attempts, in our laboratory, to anesthetize *Helisoma duryi* in preparation for surgery have been most frustrating. When placed into an unhospitable environment, such as a dilute solution of sodium pentobarbital, the animals retracted into their shells secreting copious mucus. In *Planorbis corneus* this problem has been solved in part by dialysing chloral hydrate into the anesthetization medium[17]. None of the aforementioned procedures produce suitable anesthesia for surgery. The method of Liebsch et al.[13] produced sufficient relaxation to conduct therapeutic injections but was inadequate for surgery. However, we were not able to modify this procedure for surgical manipulations.

To anesthetize *Helisoma*, snails (10–20), taken from 6-month-old age synchronized populations, were placed in 500 ml of gently aerated dechlorinated tap water (DCW). Sodium pentobarbital (Abbott Labs: Nembutal) was introduced in a dropwise manner over a period of 10–12 h yielding a final concentration of 0.8% (v/v) (fig.). A drawn-out microlitre pipette, affixed to a 10-ml syringe body was used as a dispensing apparatus. Animals were kept in this medium for approximately 4 more hours. Tentacular response to tactile stimulation and the absence of foot retraction were used as criteria for surgical suitability. Eversion of the penal complex (verge and preputium) indicated that anesthesia had progressed too far and led to poor recovery. Recovery from anesthetization was best achieved by placing the animals in 4L aerated DCW rather than in running water. Successful recovery from initial anesthesia was in the 80–100% range while recovery from subsequent (2nd and 3rd) anesthetization was 100%.

Although effective, this procedure suffers from several disadvantages. Firstly, it requires 16 h for anesthesia and secondly, a high concentration of sodium pentobarbital is required (3.2 mM). The third problem is that anesthesia has a marked effect on the snails' circulation. The pedal sinus becomes engorged with hemolymph and blood loss is appreciable during surgery. The application of a fine stream of N_2 (g) to the region of cautery minimizes blood loss, improving post-surgery survival rates.

In vertebrates, barbiturate general anesthetics are believed to act on the central nervous system at the level of the ascending reticular formation and/or the cerebral cortex[18,19]. Due to their lipid solubility, the neutral forms (unionized) of anesthetics enter membranes most easily[18-21]. At the cellular level, barbiturates are believed to act by depressing the activities of excitable tissues[18]. This is achieved by depressing excitatory as well as enhancing inhibitory synaptic transmission[19,22,23]. At the molecular level, barbiturate somnifacients bind axonal membrane proteins and modify their electrogenic activity, leading to anesthesia[19,20]. Although less sensitive than the central nervous system, components of the peripheral nervous system respond to hypnotics in a manner similar to that produced by tertiary amine local anesthetics[18].

Since pentobarbital has a pKa 8.11[24,25] precisely 50% would be in the effective unionized form at pH 8.11[26]. As the pH de-