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.

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2 Fries, N., Trans. Br. mycol. Soc. *60* (1973) 1.
3 Griffin, G.J., Hora, T.S., and Baker, R., Can. J. Microbiol. *21* (1975) 1468.
4 Hora, T.S., and Baker, R., Trans. Br. mycol. Soc. *59* (1972) 491.
5 Johri, K., Johri, B.N., and Saksena, S.B., Pl. Soil. *43* (1975) 347.
6 Lockwood, J.L., Biol. Rev. *52* (1977) 1.
7 Mitchell, C.P., and Dix, N.J., Trans. Br. mycol. Soc. *65* (1975) 259.

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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.

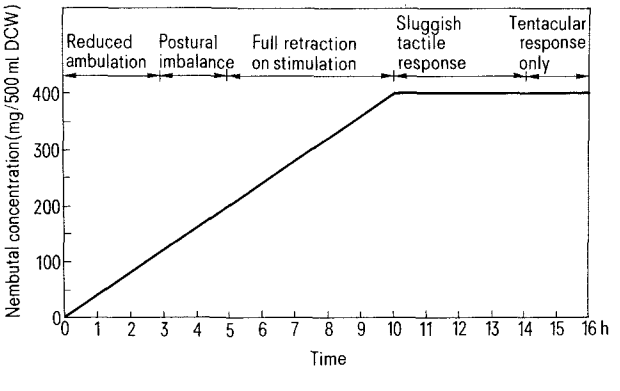
Anesthesia of gastropods has been employed for relaxation prior to fixation[2-11], injection and/or hemolymph sampling[12,13], and surgery[7,14-16]. Variation in the response to anesthetics has been reported not only between genera but also between species of the same genus. Of the numerous agents employed 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-



Summary of parameters influencing anesthesia and recovery

Medium (500 ml)	Initial pH	Final pH	Δ pH	Δ Time (h)	Time to crawling (h)	Time to feeding (h)	Time to egg laying (h)
a) Experimental (Nembutal present at a final concentration of 0.8%)							
DCW	7.00	8.49	1.49	15.45	12	21	36
0.01 M Trizma	7.00	7.63	0.63	15.25	3	5	23
0.02 M Trizma	7.01	7.69	0.68	14.00	3	4	24
0.05 M Trizma	7.00	7.52	0.52	16.00	3	8	30
b) Controls (Nembutal absent)							
DCW	7.00	8.49	1.49	13.45			
0.01 M Trizma	7.05	8.69	1.64	18.25			
0.02 M Trizma	7.00	7.54	0.54	16.50			
0.05 M Trizma	7.00	7.40	0.40	14.00			

creases the percentage of undissociated pentobarbital would increase favoring membrane penetration. Aerated DCW, at an initial pH 7.0 stabilizes within 2 h at pH 8.4–8.5. This phenomenon is also observed without aeration over a period of 15 h. At pH 8.5 approximately 70% of the soporific would be in the less effective ionized form. In order to favor membrane diffusion by pentobarbital a Trizma (Sigma Chemical Co.) buffer system was employed. Three molarities of buffer were investigated and evaluated with respect to ΔpH, anesthetization time, recovery time to crawling, feeding and oviposition (table). Animals maintained in Trizma buffered DCW were found to continue normal activities for several days, showing signs of discomfort only in the 0.05 M solution. Using this system, ΔpH over the course of anesthetization could be kept to one third of that using DCW alone. Recovery time leading to crawling and the onset of feeding was reduced to $\frac{1}{3}$ and $\frac{1}{4}$ respectively. Little difference was seen between using Trizma buffer at 0.01 M, 0.02 M or 0.05 M so the least concentration was adopted. At the initial pH 7.0 approximately 92% of the pentobarbital is present in the unionized form, while at the final pH 7.6 about 76% remains unionized, favoring efficient anesthetic activity.

The reduction in recovery time, when using a Trizma buffered anesthesia medium is difficult to interpret. The limitation of ΔpH may reduce osmotic stress. Alternatively, prolonged exposure to ionized pentobarbital may be more toxic to the animal than is the unionized form. Although recovery time is enhanced we were unable to reduce the anesthetization time.

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2 Van Der Schalie, H., Am. Midl. Nat. *50* (1953) 511.
3 Van Eeden, J.A., Proc. malac. Soc. *33* (1958) 64.
4 McCraw, B.M., Nature *181* (1958) 575.
5 Carriker, M.R., and Blake, J.W., Nautilus *73* (1959) 16.
6 Smith, E.H., The Veliger *4* (1961) 52.
7 Runham, N.W., Isarankura, K., and Smith, B.J., Malacologia *2* (1965) 231.
8 Beeman, R.D., Pubbl. Staz. zool. Napoli *36* (1968) 267.
9 Bailey, T.G., Experientia *25* (1969) 1225.
10 Meier-Brook, G., Malac. Rev. *9* (1976) 115.
11 Barker, G.M., The Veliger *24* (1981) 76.
12 Michelson, E.H., Trans. Am. microsc. Soc. *77* (1958) 316.
13 Liebsch, M., Becker, W., and Gagelman, G., Comp. Biochem. Physiol. *59A* (1978) 169.
14 Joosse, J., and Lever, J., Proc. K. ned. Akad. Wet. *C62* (1959) 145.
15 Lever, J., Jager, J.C., and Westerveld, A., Malacologia *1* (1964) 331.
16 Townsend, C.R., Anim. Behav. *21* (1973) 549.
17 Lichtensteiger, W., Felix, D., and Hefti, F., Brain Res. *170* (1979) 231.
18 Harvey, S.C., in: The pharmacological basis of therapeutics, p. 102. Ed. L.S. Goodman and A. Gilman. MacMillan, New York 1975.
19 Richards, C.D., Trends Neurosci. (1980) 9.
20 Maynert, E.W., in: Drill's pharmacology in medicine, p. 250. Ed. J.R. DiPalma. McGraw-Hill, New York 1971.
21 Narahashi, T., Frazier, D.T., Deguchi, T., Cleaves, C.A., and Ernau, M.C., J. Pharmac. exp. Ther. *177* (1971) 25.
22 Weakly, J.N., J. Physiol. *204* (1969) 63.
23 Richards, C.D., J. Physiol. *227* (1972) 749.
24 Ritschel, W.A., in: Perspectives in clinical pharmacy, p. 286. Eds D.E. Francke and H.A.K. Whitney, Jr. Drug Intelligence Publications, Hamilton, Illinois 1972.
25 Fasman, G.D., Handbook of biochemistry and molecular biology: Physical and chemical data VI. CRC Press, Cleveland 1976.
26 Sjoqvist, F., Borga, O., and Orme, M.L.E., in: Drug treatment: Principles and practise of clinical pharmacology and therapeutics, p. 1. Ed. G.S. Avery. Adis Press, Sydney, Australia.

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Identification of rooting depth and measurements of plant root activity in situ and in toto under field conditions using a gamma probe technique

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Summary. A technique for measuring root activity of agricultural crops in totality in situ under field conditions has been developed for the first time. The method essentially consists of measuring the activity emanating from roots after plant injection of a gamma radiating isotope using a probe. This also helps continuous monitoring of root growth changes over time.

Breeding of crop varieties with deep and voluminous root system ensures efficient utilization of scarce agricultural inputs. Research on the identification of such varieties has been greatly handicapped by the lack of suitable techniques for studying the root distribution. In recent years, radio-isotope techniques have become available for measuring root activity. Extensive investigations on the root activity distribution have been carried out using ^{32}P[2-7] and ^{86}Rb[8-12] by an injection tech-