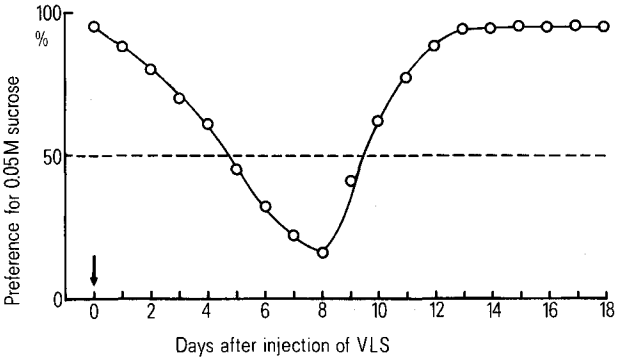


Fig. 3. Change in preference for 0.05 M sucrose after VLS. The preference is expressed as

$$\frac{\text{sucrose intake}}{\text{total intake of sucrose and water}} \times 100.$$

Sucrose intake values were obtained from the corrected curve in figure 2, A. The horizontal line indicates a 50% water intake level that separates preference from aversion.

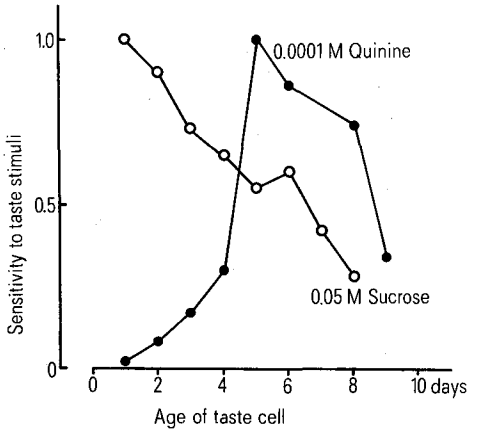


Fig. 4. Estimated relationship between age of taste cells and their sensitivity to taste stimuli. In ordinate, maximum sensitivity of taste cells to either stimulus is given as 1.0. The sensitivity to quinine was calculated from the quinine intake curve for 2.5 mg VLS in figure 1.

relation between the taste nerve response to sucrose below 0.1 M and the intake of sucrose in control rats. In contrast, there was an inversely proportional relation between the taste nerve response to quinine below 0.001 M and the intake of quinine. The taste nerve response can be replaced with the taste cell response because there is a proportional relation between them[9]. These lines of evidence[10] suggest that the alteration of taste cell sensitivity by aging is closely correlated with changes in taste preference and aversion.

Based on the average life span of 10 days in rat taste cells[2], we assume that the population ratios of taste cells are linearly reduced with aging: number of 1-day-old taste cells is taken as 1.0, 5-day-old cells 0.6 and 10-day-old cells 0.1. From the corrected sucrose intake curve in figure 2, A, the average sucrose response per taste cell in a t-day age may be expressed as $(I_t - I_{t+1})/P_t$, where $I_t$ and $I_{t+1}$ are amount of sucrose intakes on day t and day t + 1 after VLS, and $P_t$ is the population ratio of t-day-old taste cells. On the other hand, from figure 1 the average quinine response per taste cell in a t-day age may be expressed as $P_t/(I_t - I_{t+1})$, because the quinine intake is in an inverse proportion to the taste cell response to quinine. Figure 4 was obtained from the above equations. It is seen that the sucrose sensitivity of taste cell reduces gradually with aging, while quinine sensitivity increases till 5 days of age, after which it falls gradually.

1 We thank Professor M. Ichioka for his encouragement during this work and Dr D. Tucker for his valuable comments on the manuscript.
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## Diurnal variations in cholinesterase activities in the slug, *Laevicaulis alte*

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*Summary.* Cyclical variations in acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) levels in foot muscle (FM) and AChE activity in central nervous system (CNS) of *Laevicaulis*, during 24-h-day, were maximal at 04.00 h and minimal at 12.00 h. But BuChE activity was 180 °C out of phase with AChE in CNS. The rhythmic trend of AChE in CNS might be due to true cholinesterase activity.

The occurrence, distribution and localization of acetylcholinesterase (AChE) in various molluscs have been demonstrated[2]. Associated with true cholinesterases, nonspecific (pseudo) cholinesterases have also been reported in molluscs[3].

Rhythmic variations in AChE, with peak periods of activity during dark h, have been found in nocturnal animals like scorpions, cockroaches and snails[4-6]. But studies on cyclical variations in pseudocholinesterases are lacking. The present investigation reports on AChE rhythm in the slug, *Laevicaulis alte*, and a spot check study on butyrylcholinesterase (BuChE) activity.

*Methods.* Adult specimens of *Laevicaulis* were collected in and around Tirupati. In the laboratory they were main-

tained in wooden boxes containing mud. The selection of time periods for experimentation was described earlier[7].

AChE and BuChE levels in CNS and FM of the slug were estimated following the method of Metcalf[8] and proteins by the method of Lowry et al.[9]. The activity levels of the enzymes were expressed as µmoles of ACh and BuCh hydrolyzed/mg protein/h for AChE and BuChE, respectively.

*Results.* The maximal AChE activity during 24-h-period of the day (12 h light:12 h dark) in CNS and FM was at 04.00 h, while the minimal activity occurred at 12.00 h (figure 1, a). At 04.00 h the level of AChE was greater by 50.0% in CNS and 39.6% in FM than the level of enzyme at 12.00 h ($p < 0.001$ for both the tissues). Further, analysis of $\chi^2$ for all 6 periods, with Friedman's 2-way analysis of variance by ranks[10] showed that null hypothesis (Ho) cannot be rejected for both CNS and FM since $\chi^2$ values fall outside the rejection zone at $p < 0.0017$ level of significance (lesser than $a = 0.01$), when $n = 3$, and $K = 6$. The average level of the enzyme during 20.00–04.00 h was higher ($p < 0.01$ for CNS and 0.005 for FM) than during 08.00–16.00 h (figure 1, b). Further, AChE activity was greater in FM than in CNS.

Spot check study of BuChE levels at 12.00 h and 04.00 h in the CNS and FM registered variations (figure 2). The pseudocholinesterase level was lower in CNS than in FM. In FM BuChE activity was 2fold higher than in CNS. BuChE level at 04.00 h was 42.4% less in CNS and 55.6% greater in FM than at 12.00 h ($p < 0.1$ for CNS and 0.01 for FM). Friedman's test[10] showed that since $\chi^2$ values for both CNS and FM fall within the zone of rejection when $n = 3$ and $K = 2$, Ho can be rejected.

*Discussion.* The observed higher level of AChE activity in FM than in CNS may be due to: a) innervation of large areas of the tissue, b) to probable presence of nerve plexus[11]; or c) to greater levels of pseudocholinesterases. The present study demonstrates that BuChE level was lower in CNS than in FM. This suggests that cyclical variations observed in AChE activity in CNS might be largely due to true cholinesterase activity. Unlike in CNS, BuChE level in FM significantly increased, proportionally with AChE.

Several physiological rhythms were correlated with running activity of the animal. Rockstein[12] stated that with motoric functions in insects, a rise in AChE activity occurs in CNS. Such correlation between AChE, electrical and locomotor activities in nocturnal animals like scorpions[13,14] cockroaches[5] and snails[6] etc. was shown. Leon and Rosenberg[15] reported a relationship between AChE and axonal conduction.

Our studies on locomotor activity of *Laevicaulis* showed that the peak period of motoric activity was between 22.00–03.00 h during dark h[16]. Thus, the cyclical variations in AChE and running activity of the animal are in phase with each other. Hence, as mentioned above, in the slug also the enzyme activity may be related to locomotor activity of the animal.

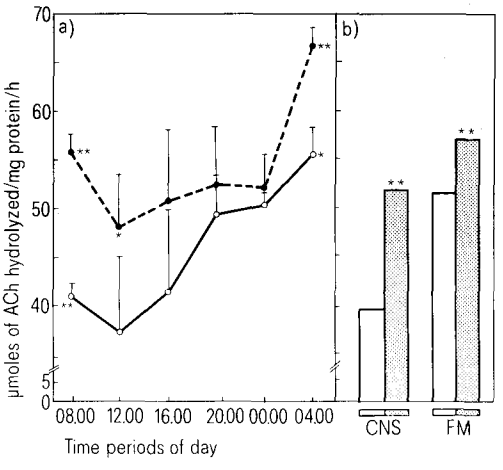


Fig. 1*a*. Cyclical variations in AChE activity in CNS (O----O) and FM (●----●) of *Laevicaulis*. Each value was the mean of 4 individual observations. For each observation, a total number of 12 animals were used. *b* Variations in AChE levels in CNS and FM of *Laevicaulis* during light (08.00–16.00 h: ▭) and dark (20.00–04.00 h: ▨) h of the solar day.

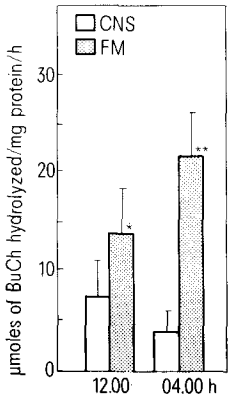


Fig. 2. Spot check study of BuChE activity in CNS and FM of *Laevicaulis* at 24.00 and 04.00 h. CNS = Central nervous system; FM = foot muscle, * = Statistically significant ($p < 0.02$), ** = Statistically highly significant ($p < 0.001$).

1 Acknowledgments. The Senior Fellowship (CSIR) awarded to TPK is thankfully acknowledged.

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