At the level of the thalamus, it has been shown that SI cerebral cortex inhibits the progress of primary impulses beyond the VPL nucleus[20,21]. It is possible that PGM stimulation inhibits this thalamic nucleus through cortical activation; such cortical activation may be produced through the ascending activating system of the brain stem. Finally, a directly inhibitory action on primary cortex is also possible through the diffuse projecting system or the specific projection of VPL through a lemniscal-extralemniscal convergence[22,23]. Of the 3 possibilities analyzed here, the first can be excluded, since this inhibitory effect was observed in both anaesthetized and conscious animals.

It is important to emphasize that the inhibition of the negative component on the cortical evoked potential was greater than that of the positive component. This observation does not support the idea of subcortical inhibition, since under these circumstances both components should have been similarly affected. It is also important that inhibition is more evident when longer periods of stimulation are applied. This finding agrees with observations by Melzack and Melinkoff[24] that electrical stimulation of central gray matter has to be applied for about 5 min for maximum analgesia. In addition, the inhibitory action we observed persisted for 2 or 3 min after stimulation was interrupted, with full recovery in 5 min. This persistence of the inhibitory action upon cortical evoked potentials is comparable to the observations on electrical analgesia in rats, performed by other investigators[2-4].

The inhibitory effects reported here may participate in the analgesic mechanism elicited by stimulation of PGM. Unfortunately we do not have confirmatory data that support such a hypothesis.

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Rhythmic variations in the isocitrate dehydrogenase activity in the scorpion, Heterometrus fulvipes (C. Koch)

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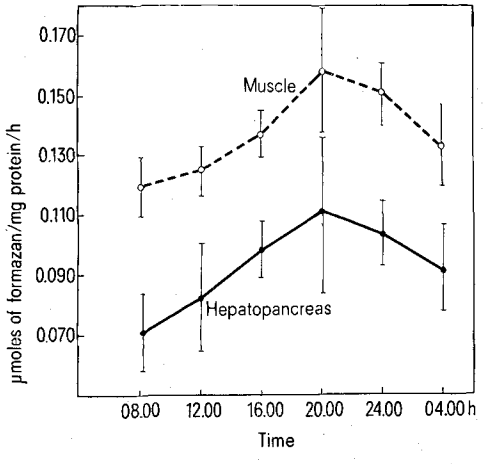
Summary. The activity of isocitrate dehydrogenase was assayed in the pedipalpal muscle and hepatopancreas of scorpion, Heterometrus fulvipes. The enzyme activity showed a circadian rhythmicity with a peak value at 20.00 h in both the tissues.

Rhythmicity in arachnid metabolism has received only limited attention. Diurnal rhythms in various activities like locomotion, poison secretion[1], neurosecretion[2], rate of heart beat, choline esterase activity in heart muscle[3], phosphorylase activity in the hepatopancreas and muscle[4], spontaneous electrical activity in the ventral nerve cord and segmental nerves[5], in the levels of metabolites like blood glucose and hepatopancreatic glycogen[6], have been reported in the scorpion, Heterometrus fulvipes. The above investigations suggest that the biological constituents (metabolites and enzymes) vary in a rhythmic manner during 24 h. This prompted us to study the isocitrate dehydrogenase activity in the pedipalpal muscle and hepatopancreas of the scorpion, Heterometrus fulvipes, as a function of the time of day. The pattern of activity of this enzyme which plays a vital role in citric acid cycle should reveal the pattern of utilization of energy sources for various activities during the course of 24 h period.

Material and methods. The details of collection and maintenance of scorpions, and sampling of tissues were described earlier[6,7]. 10% (w/v) homogenates of tissues were prepared in 0.25 M ice-cold sucrose and centrifuged at 2,500 rpm for 15 min; 0.5 ml of each supernatant (containing 50 mg of tissue) was assayed for the isocitrate dehydrogenase (EC.1.1.1.41) activity level by the method of Kornberg and Pricer[8] with the following modifications.



Isocitrate dehydrogenase activity in the scorpion, Heterometrus fulvipes, as a function of the time of day. Each point represents the mean of 6 estimations. The vertical bars above and below the points indicate the SD limits. The animals were maintained in the laboratory under normal (12 h light/12 h darkness) conditions. The day and night temperatures during the experiment were 36 ± 1 °C and 28 ± 1 °C respectively.

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The reaction mixture of 2 ml contained 20 μmoles of DL-isocitrate; 100 μmoles of phosphate buffer (pH 7.4); 4 μmoles of INT (2-p-iodophenyl-3-nitrophenyl tetrazolium chloride); 10 μmoles of $MgCl_2$; 0.2 μmoles of ADP and 0.2 μmoles of NAD^+. The reaction was initiated by the addition of 0.5 ml of supernatant. The control reaction mixture received 0.5 ml of sucrose in the place of supernatant solution. After an incubation of 30 min at 37 °C, the reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazan formed due to reduction of the dye was extracted into 5 ml of toluene for overnight in cold, and colour was read in UV spectrophotometer (Hilger & Watts, England) at 495 nm using silica cuvette of 10 mm path length. Individual zero time controls were maintained for all the samples by the addition of glacial acetic acid to the reaction mixture prior to the addition of the enzyme and incubation. The enzyme activity was expressed as μmoles of formazan/mg protein/h. Protein levels were determined by the method of Lowry et al.[9]. The data was subjected to statistical processing according to standard procedures (Pillai and Sinha)[7].

Results and discussion. The activity levels of isocitrate dehydrogenase were found to range from 0.120 ± 0.014 to 0.158 ± 0.020 μmoles of formazan/mg protein/h in the pedipalpal muscle and 0.071 ± 0.013 to 0.111 ± 0.026 μmoles of formazan/mg protein/h in hepatopancreas (figure). In both the tissues, the maximal activity was recorded at 20.00 h and minimal activity was recorded at 08.00 h. In both tissues the difference between the maximal (20.00 h) and minimal (08.00 h) was significant ($p < 0.001$ for muscle; $p < 0.001$ for hepatopancreas). But the pattern of rise and fall in isocitrate dehydrogenase activity in between these times was different in the 2 tissues studied. In both the tissues, eventhough the peak enzyme activity is found at 20.00 h, the enzyme activity remains relatively high between 16.00 h and 04.00 h.

The higher isocitrate dehydrogenase activity in the muscle perhaps reflects increased channeling of the substrates to the citric acid cycle for energy requirements during muscle contraction. Hepatopancreas is known to be the main organ for storage of nutrients. Synthesis and break down of metabolites are related to the metabolic needs of the animal. The scorpion is a nocturnal animal and shows a significant increase in locomotor activity at night[1]. The metabolic rate is also high between 16.00 h and midnight (24.00 h) with a peak around 20.00 h[1]. The necessary energy is perhaps made available through increased metabolic degradation of glycogen, as evidenced by increased phosphorylase activity[4] and blood glucose[6]. The nocturnal peak of the isocitrate dehydrogenase activity at 20.00 h may signify increased channeling of substrates through the Krebs cycle to sustain the raised energy requirements due to the increased locomotor activity, involving higher rate of muscle contraction of the animal during night.

It is known in mammals that epinephrine induces greater production of cyclic-AMP in muscle, which leads to the acceleration of breakdown of glycogen in liver and muscle[11]. Circadian rhythm in the neurosecretions in the scorpion has also been reported[2]. It is probable that the active principle from scorpion neurosecretory system with epinephrine-like action is responsible for increased channeling of substrates to the Krebs cycle for mobilization of energy through the activation of isocitrate dehydrogenase.

Thus the peak activity of the isocitrate dehydrogenase activity at 20.00 h, coinciding with the nocturnal habit of the scorpion, appears to be significant in view of the raised energy requirements to sustain the nocturnal increase of locomotor activity.

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Measurement of the biliary tree volume in the rat under T_m-conditions

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Summary. Biliary tree capacities determined under T_m-conditions using ^{35}S-sulfobromophthalein sodium, ^{35}S-sulfobromophthalein glutathione, and ^{14}C-taurocholate sodium as markers exceeded markedly the biliary volumes reported until now. This may have been caused at least partly by a more homogenous distribution of the marker substance throughout the liver parenchyma in contrast to simple bolus injection studies.

About 15 years ago, Barber-Riley[1] estimated the capacity of the rat biliary as the volume of bile collected between the time of i.v. injection of sulfobromophtalein and the time at which the concentration of this dye in bile reached one-half maximal excretion rate. However, it now appears that Barber-Riley's method may give erroneous estimates of capacities in that a time lag occurs between the i.v. administration of the dye and its excretion into the bile. Häcki and Paumgartner[2] have largely overcome this transit time problem by using ^{14}C-taurocholate as a marker, and they reported nondistended biliary tree capacities of a rat liver weighing 10 g of about 23 μl. The retrograde push-back technique of Peterson et al.[3] also does not contain a transit time artifact and gives 37 μl as the estimate for biliary tree capacities in the distended state in contrast to 121 μl found by Barber-Riley[1]. Although different methods were used to determine the biliary tree in its nondistended or distended state, they are based on the same theoretical consideration that the dye injected is distributed or washed out homogenously throughout the biliary system, and thus may permit measurement of the biliary tree volume. This assumption has not been proved yet. An approach to determine the biliary tree capacity under T_m-conditions was introduced using ^{35}S-sulfobromomophthalein, ^{35}S-sulfobromophthalein glutathione, and ^{14}C-taurocholate as markers.

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