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CIRCADIAN RHYTHMIC ACTIVITY OF ISOCITRATE DEHYDROGENASE IN THE SLUG, *LAEVICAULIS ALTE* (FERRUSAC, 1821)

ALTHOUGH considerable amount of information concerning different types of rhythms in molluscs[1–4] is available, very little work was done on the rhythmic activity of enzymes in these animals. Slugs are shown to be nocturnal animals[5] and hence there could be corresponding variations in the various physiological processes of these organisms. Enzymes like phosphorylase[6], alkaline and acid phosphatase[7], lipase[8] and metabolites like hepatopancreatic glycogen, blood glucose[9] were shown to undergo regular rhythmic changes in the slug. In view of above, it is proposed to assay the activity pattern of isocitrate dehydrogenase a key enzyme in the citric acid cycle, in the hepatopancreas and foot muscle of the slug, as a function of time of the day. The activity pattern of this enzyme during the course of 24h period may reflect the pattern of utilization of energy sources to sustain the energy needs of the animal for various activities.

The details of collection and maintenance of slugs and sampling of tissues were described earlier[6]. Homogenates of tissues (10% w/v) were prepared in 0·25 M ice-cold sucrose and centrifuged at 2500 rpm for 15 min; 0·5 ml of each supernatant (containing 50 mg of the tissue) was assayed for the isocitrate dehydrogenase (EC 1.1.1.41) activity level by the method of Kornberg and Pricer[10] as modified by Mastanaiah *et al.*[11]. The reaction mixture of 2 ml contained 20 μ moles of DL–isocitrate; 100 μ moles of phosphate buffer (pH 7·4); 4 μ moles of INT (2-*p*-iodophenyl-3-nitrophenyl tetrazolium chloride); 10 μ moles of $MgCl_2$, 0·2 μ moles of ADP and 0·2 μ moles 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 place of supernatant solution. After an incubation for 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 (overnight in cold) and the colour was read in UV spectrophotometer at 495 nm using silica cuvette of 10 mm path length. The 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 μ moles of formazan/mg protein/h. Protein levels were determined by the method of Lowry *et al.*[12]. The data were subjected to statistical processing according to standard procedure (Pillai and Sinha)[13].

The present study shows a typical clock connected rhythm in the ICDH activity, with maximal activity around 00·00 h and minimal activity around 12·00 h of the day in both the tissues. The enzyme ranges from $0·125 \pm 0·015$ to $0·165 \pm 0·011$ μ moles of formazan/mg protein/h in the foot muscle and $0·081 \pm 0·010$ to 0·132–0·012 μ moles of formazan/mg protein/h in hepatopancreas. In both the tissues the difference between the maximal (00·00 h) and minimal (12·00 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 intervals were different in

the two tissues studied. In both the tissues, even though the peak enzyme activity is found at 00·00h, the enzyme activity remains relatively high between 20·00 h and 08·00 h. The greater ICDH activity in foot muscle over the hepatopancreas may reflect the utilization of energy sources for muscle contraction. Similar type of diel rhythms have been reported in the enzymatic activities of snail[14] and the slug. It was also reported that the rate of heart beat and locomotion in the slug followed a regular diurnal rhythm[4]. The increased locomotor activity during night in the slug requires energy in the form of ATP molecules, and consequently there is an enhancement of mitochondrial enzymes which in its turn may result in elevated activity levels of isocitrate dehydrogenase.

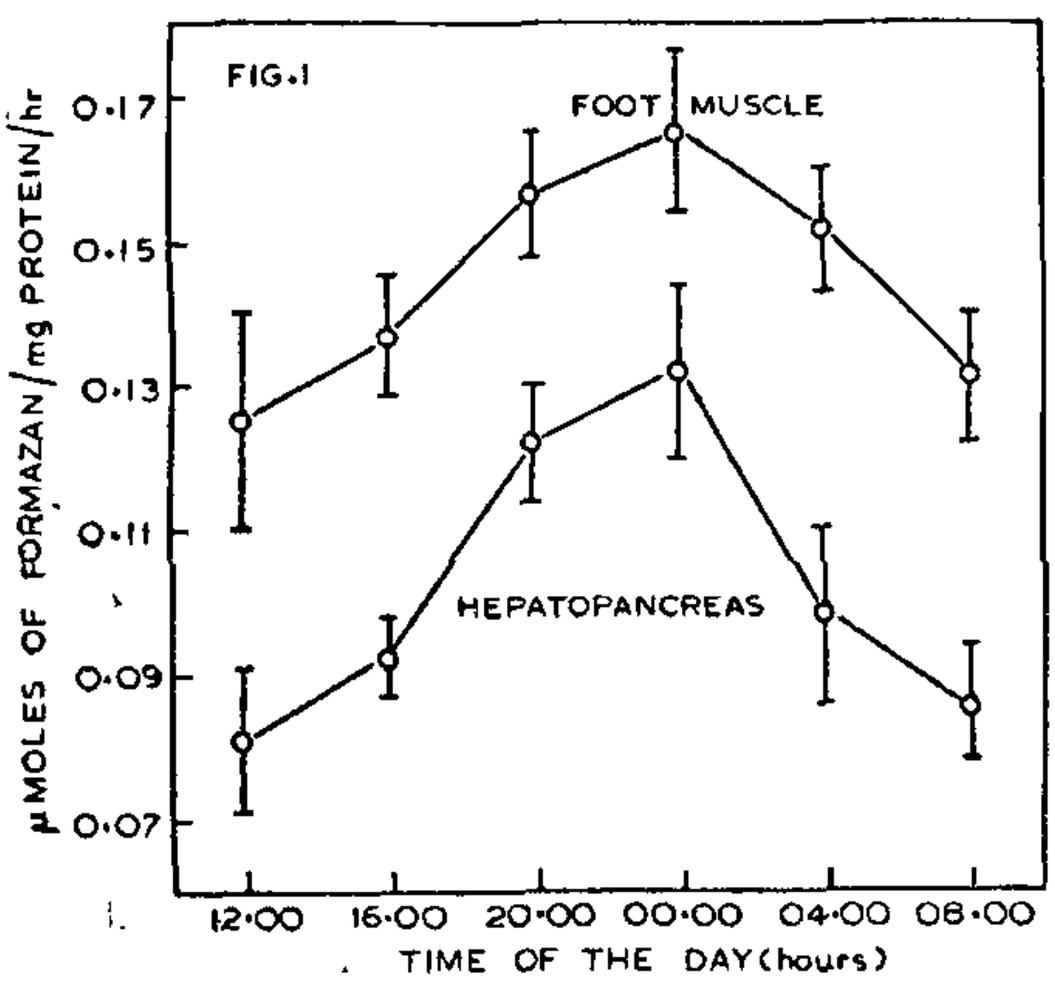

FIG. 1. Isocitrate dehydrogenase activity in the slug, *Levicaulis alte*, as a function of the time of the day. Each point represents the mean of 6 estimations. The vertical bars above and below the points indicate SD limits. The animals were maintained in the laboratory under normal (12 h light/12 h darkness) conditions. The day and night temperatures during the experiment were 33 ± 1 and 25 ± 1° C respectively.

The higher activity level of ICDH around midnight in the hepatopancreas may be due to the higher energy needs for the increased physiological activities around that period. Further the increase in glycogenolysis as evinced by increase in the activity level of phosphorylase[6] and blood glucose[9] and an increase in lipolysis, as evinced by the increase in the activity level of lipase[8] in the hepatopancrease around midnght may probably due to the increase of the enzymes in Krebs cycle. The present finding of nocturnal elevation of ICDH in the hepatopancreas is in close agreement with the above findings.

Thus the peak activity of the isolcitrate dehydrogenase at 00·00 h, coinciding with the nocturnal habit of the slug, appears to be significant in view of the raised energy requirements to sustain the nocturnal increase of locomotor activiiy.

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CHAEMOARCHITECTONICS OF THE PROSTATE GLANDS OF TWO DIGENETIC TREMATODES

ALTHOUGH sufficient literature is available on the histophysiology of the female reproductive organs of digenetic trematodes[1,3,4,6,9,10], relatively very little information is available on the male reproductive organs and in particular of prostate glands. The ultrastructure of the latter has been described only recently[11]. The present investigation concerns histochemical localization of certain enzymes and non-