CLOCK-CONNECTED RHYTHMICITY IN PHOSPHORYLASE ACTIVITY IN THE SLUG, LAEVICAULUS ALTE (FERRUSSAC 1821)

Rhythmic variations have been noted in many animals for numerous biological parameters\(^1\). Although considerable amount of information concerning different types of rhythms in molluscs\(^2\)-\(^5\) is available, very little work was done on the rhythmic activity of enzymes in these animals. Slugs are shown to be nocturnal animals\(^6\) and hence there could be corresponding variations in the various physiological processes of these organisms. In view of this it is proposed to assay the activity pattern of phosphorylase, a key enzyme in glycogenolysis, in the hepatopancreas and foot muscle of the slug, as a function of time of the day. The activity pattern of this enzyme may reflect the pattern of utilization of carbohydrate energy sources for various activities during the course of 24 hr period.

The details of collection and maintenance of slugs were described earlier\(^7\). The hepatopancreas and foot muscle were isolated (tissues from two animals were pooled to represent a single sample) in cold (5° C) from live slugs of similar size, at regular interval of 4 hr (two samples at each time).

The tissues were weighed and 2% (W/V) homogenates were prepared in an aqueous medium containing 0.037 M ethylene diamine tetracetic acid (pH 6.5) and 0.1 M sodium fluoride (pH 6.5) as recommended by Guillory and Mommaerts\(^8\). It was centrifuged at 2500 rpm for 10 min and the supernatant was used for the enzyme assay. The activities of phosphorylase 'a' (active) and 'ab' (total) were assayed in the absence and presence of AMP respectively, by the method of Corn et al.\(^9\). Inorganic phosphate was estimated by the method of Fiske and Subba Row\(^10\) and the enzyme activity was expressed as μmoles of Pi/mg protein/hr. Protein content was estimated by the method of Lowry et al.\(^11\).

The present study demonstrates a typical clock connected rhythm in the phosphorylase activity with maximal activity at 00:00 hr and minimal activity at 12:00 hr of the day (Fig. 1). Total phosphorylase ranges from 71.3 to 93.8 μmoles of Pi/mg protein/hr in the hepatopancreas and from 74.5 to 88.7 μmoles of Pi/mg Protein/hr in foot muscle. Active phosphorylase ('a') also followed a similar trend in both the tissues. It varies from 15.5 to 29.5 μmoles of Pi/mg protein/hr and from 20.8 to 32.5 μmoles of Pi/mg protein/hr in the hepatopancreas and foot muscle respectively. Even though the total phosphorylase ('ab') activity in foot muscle is slightly higher at the minimal activity periods (12:00 and 16:00 hr), than the hepatopancreas, the range is essentially similar. But the pattern of rise and fall in phosphorylase 'ab' is sudden and sharp in the hepatopancreas as compared to the foot muscle. The greater phosphorylase ('a') activity in foot muscle over the hepatopancreas may reflect glucose utilization for muscle contraction.

**Fig. 1.** Clock connected rhythm in phosphorylase activity in the hepatopancreas and foot muscle of the slug. Values expressed at each time interval are mean ± S.D. of six observations.

Similar type of diel rhythms have been noticed in the enzymatic activities of the snail\(^12\) and the slug\(^7\). It is also found that the rate of heart beat and locomotion in the slug followed a regular diel rhythm\(^5\). It has been shown that the blood glucose level is maximum and hepatopancreatic glycogen level was minimum at 00:00 hr indicating the mobilization of glucose from hepatopancreas to the blood\(^13\). The maximal phosphorylase activity in the hepatopancreas and foot muscle in the present study at 00:00 hr may be correlated to the minimal hepatopancreatic glycogen content and maximal blood sugar level. After 00:00 hr, phosphorylase activity gradually decreases as there would be enough glucose in the blood due to post-prandial absorption. The excess glucose may, probably, be transported to the hepatopancreas for glycogenesis. This is evident from the fact that the glycogen level in the hepatopancreas is progressively increased after 00:00 hr reaching its maximum at 12:00 hr of the day. Based on these observations, it may be suggested that the high phosphorylase activity in the slug during darker periods at and around 00:00 hr might be providing the necessary glucose, through
phylogenesis, required for the energetic needs of various physiological activities of the animal.

The authors thank Prof. K. S. Swami for providing facilities. Financial assistance rendered by ICMR (DCR), UGC (VJ) and CSIR (KS) is gratefully acknowledged.

Dept. of Zoology, D. CHANDRA SEKARA REDDY.
S.V. University, V. JAYARAM.
Tirupati 517 502, K. SOWJANYA.
India, B. PADMANABHA NAIDU.


CONSTITUTIVE HETEROCHROMATIN IN THE INDIAN BUSH RAT, GOLUNDA ELIOTTI (GRAY)

The chromosomes of the Indian bush rat, Goluma elliotti (Gray) have been studied by Mathew4 who reported the diploid number as 52. Later, Mittal & Kaul5 reported the diploid number for this species from Ludhiana (Punjab) as 50. We have studied the chromosomes of Goluma elliotti (Gray)2 collected near Mysore and our observations are at variance with those of the earlier reports.

Twelve individuals (6 males and 6 females) were collected from the environs of Kadakola village (8 kms. from Mysore, S. India). Bone marrow, spleen, intestinal epithelium and testis were utilized for chromosome preparations. Spleen, liver and kidney cells were directly fixed in acetic acid-methanol for sex

chromatin screening. G-banding was done according to the technique of Sumner7.

The diploid number was 54. The chromosomes were measured and classified according to the system of Levan et al6. The karyotype (Fig. 1) consists of one

pair of small metacentric and twenty-five pairs of telocentric chromosomes. The X-chromosome is sub-metacentric and measures 7.63% of the haploid genome; it is thus larger than the 'original' type according to the classification of Ohno et al6. The Y-chromosome is telocentric and measures 3.80% of the haploid complement. In C-banding (Fig. 2) all

FIG. 1. Karyotype of Goluma elliotti (Gray), male, 2n = 54.

FIG. 2. C-banded metaphase of Goluma elliotti, male.

the autosomes irrespective of their size showed dark and clear bands in the centromeric regions. In addition, heterochromatin was also seen at the telocentric regions of a few autosomes. The sub-metacentric