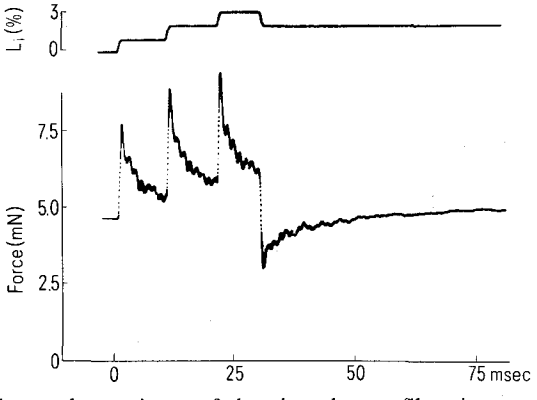
Note that the first stretch induces an immediate increase in tension which is followed by a rapid decline of tension which, within ca. 10 msec, leads almost to the value obtained before stretch. The transients observed after 2 further steps were very similar to the first one. The subsequent release produced a transient tension drop comparable to the immediate tension increases after the stretches. This immediate phase is followed by a recovery of tension up to the level observed before the first stretch, i.e. the tension remains the same while the muscle length is increased by 2% L_i.

To prove that the results obtained are due to cross bridge activity, and not only to passive elastic properties of the fibres, we performed the same experiment in relaxing solution. The tension responses observed were only about 10% of those obtained in the contracting state.

Discussion. It might be argued that detachment alone is responsible for the quick phase. One would then expect the number of attached cross bridges to become smaller with each stretch. But the elastic increase of tension induced during each stretch, and the elastic decrease in tension induced during the release, are more or less of the same amplitude. This indicates that the stiffness and consequently (cf. Huxley and Simmons[2]) the net number of attached cross bridges is almost the same at the end of each quick phase.



Triple stretch experiment of glycerinated psoas fibres immersed in a saline containing 15 mM MgATP pCa 4.9 (pH 6.7, I = 0.1 M, 23 °C). Upper trace = length change; lower trace = force developed by 2 × 5 fibres (see 'methods').

Huxley and Simmons[2] proposed that a single cross bridge is displaced by ~ 10 nm on rotation due to the force generating process. Assuming that cross bridges do not detach during and after the 1% L_i length step, the bridges ought to rotate from an acute angle into a perpendicular position as proposed by Huxley and Simmons[2] (cf. also Julian et al.[9]). However, if they remained attached, a 2nd (and 3rd) stretch should be unable to induce a quick tension decay due to cross bridge rotation. This prediction was not fulfilled in our experiments. On the contrary, a pronounced quick phase was observed after the second and even after the 3rd 1% L_i stretch, although the time between the length changes was only 10 msec.

We therefore propose that additional to crossbridge rotation the decline of tension following large stretches is attributable to detachment of cross bridges (with discharge of their elastic elements), and reattachment of cross bridges in a new (slipped) position. This process requires that attachment and detachment occur within 5 msec, i.e. at a rate greater than the known rates of ATP splitting. The postulated attachment and detachment of cross bridges is unlikely to be associated with ATP splitting, as in the case of complete cross bridge cycles. Instead they may reflect the existence of a rapid equilibrium of detached and attached bridges of the type recently proposed by White and Taylor[10] (cf. also Güth and Kuhn[11]).

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Circadian rhythmicity in phosphorylase activity and glycogen content in the heart muscle of the scorpion, *Heterometrus fulvipes*, C.L. Koch

V. Jayaram[1], D. Chandra Sekara Reddy and B. Padmanabha Naidu

Department of Zoology, S.V. University, Tirupati-517502 (India), 15 August 1977

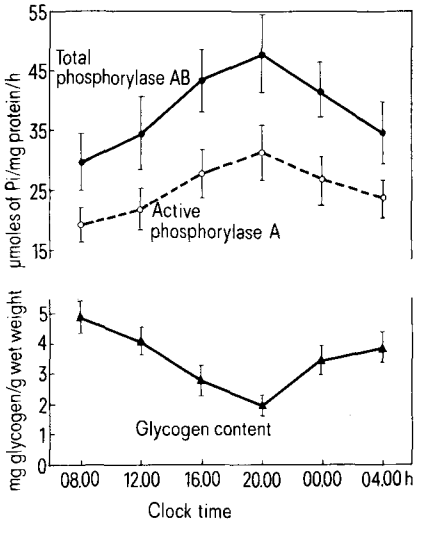
Summary. Maximal activity levels of phosphorylase A and AB at 20.00 h alternate with minimal levels at 08.00 h of the day, while the glycogen content exhibited a reverse trend in the heart of the scorpion, *Heterometrus fulvipes.*

Earlier investigations on the physiology of the scorpion, *H. fulvipes* showed the presence of rhythmic variations in locomotion[2], rate of heart beat[3], electrical activity of the ventral nerve cord[4], enzymatic activities[3,5,6] and in the levels of blood glucose and hepatopancreatic glycogen[7]. Therefore an attempt has been made to see whether such changes would also occur in phosphorylase activity and glycogen content of the heart. The activity pattern of phosphorylase, which is involved in glycogen breakdown, may reflect the pattern of utilization of carbohydrate energy sources in the various metabolic pathways during the course of 24-h period.

Material and methods. Scorpions, collected from the local hilly terrain, were maintained in glass vivaria containing

moist soil, with 12 h cool (23±1 °C) dark and 12 h warm (28±1 °C) light, and they were fed ad libitum on cockroaches. Hearts were isolated from scorpions of similar size (2 hearts were pooled to represent a single sample) in cold (5 °C) at regular interval of 4 h (2 samples at each time). The activities of phosphorylase A (active) and AB (total) were estimated in the absence and presence of AMP respectively by the method of Cori et al[8]. A 2% (w/v) homogenate was prepared in an aqueous medium containing 0.037 M ethylene diamine tetraacetic acid (pH 6.5) and 0.1 M sodium fluoride (pH 6.5), as recommended by Guillory and Mommaerts[9]. The inorganic phosphate was estimated by the method of Fiske and Subba Row[10], proteins by the method of Lowry et al.[11] and glycogen by the method of Kemp et al.[12]. The experiment was repeated for 3 consecutive days to see whether the pattern remained the same. The data was subjected to statistical treatment according to standard procedures (Pillai and Sinha)[13].

Results and discussion. The figure shows that phosphorylase A and AB exhibited parallel circadian variations (maximum at 20 h and minimum at 8 h) where as glycogen was inversely correlated. In both cases the difference between maximal and minimal levels was significant ($p<0.001$). There is a high degree of positive correlation between phosphorylase A and AB ($r=0.99$), and a high degree of inverse correlation between glycogen content and phosphorylase A ($r=-0.97$) or AB ($r=-0.96$). Even though the activity of total phosphorylase is higher than the active phosphorylase, the pattern of rise and fall is basically similar during the course of the 24-h period. The mean activity of phosphorylase A and AB is greater in the dark phase than in the light, while glycogen showed an inverse relationship (table).



Rhythmic variations in phosphorylase activity and glycogen content in the heart muscle of the scorpion, *H.fulvipes*. Values expressed at each time are mean ± SD of 6 observations.

Phosphorylase (A and AB) levels and glycogen content in the scorpion heart during light and dark phases of the day

Phase of the day	Phosphorylase A[a]	Phosphorylase AB[a]	Glycogen[b]
Light phase (08.00-16.00 h)	23.34 ± 4.09 (18)[c]	36.3 ± 6.24 (18)[c]	4.00 ± 1.00 (18)[c]
Dark phase (20.00-04.00 h)	27.60 ± 4.19 (18)[c]	42.00 ± 5.82 (18)[c]	3.16 ± 1.06 (18)[c]
% Change	18.25	15.70	21.00
t value	4.21[d]	5.7[d]	3.36[d]

[a] µmoles of Pi/mg protein/h; [b] mg glycogen/g wet weight of the tissue; [c] contain number of individual observations; [d] $p<0.001$.

The pattern of variation in phosphorylase activity and glycogen content of the scorpion heart is of interest in the context of rhythmic variations observed in oxygen consumption[2], neurosecretion[14], alkaline phosphatase activity[5], rate of heart beat and cholinesterase activity[3] in the same species. Similar rhythms have also been reported in liver glycogen, blood glucose[15] and plasma free fatty acids[16] in other species. Scorpion is a nocturnal animal, actively moving around at night. Locomotion and heart rate were found to be high at 20.00 h which reflects the elevated metabolic rate of the animal. The high phosphorylase activity at 20.00 h (figure) might be furnishing necessary glucose through glycogenolysis to meet the high energy requirements of the heart. In the present study, as shown by the results, the variations in phosphorylase activity bear an inverse relation to the corresponding variations in glycogen content, while the former shoots up to a maximum, the latter declines to a minimum and vice versa.

It has been shown that the central nervous system of scorpions produces 2 different neurohormones out of phase with each other[4,17,18]; and they seem to affect the acetylcholine synthetic and energy yielding mechanisms of the heart leading to diurnal variations. It is probable that these 2 neurohormones are responsible for the observed variations in phosphorylase system of the heart muscle of the scorpion. Thus the high activity levels of phosphorylase during the dark phase of the day (table), coinciding with the nocturnal habit of the scorpion, appears to be significant in view of the elevated heart rate and other physiological processes.

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