Daily Fluctuation in the Blood Sugar Concentration of the House Cricket, Gryllus domesticus L.

Abstract. *The disaccharide trehalose is the main carbohydrate constituent in the hemolymph of the house cricket,* Gryllus domesticus *L. At different times of day the blood sugar in the hemolymph of crickets showed a fluctuation in trehalose concentration, which reached a peak value about 3 hours before dawn.*

There have been numerous studies of daily fluctuations in the concentrations of various metabolites in man and other mammals, but there is a noticeable scarcity of information on daily metabolic fluctuations in insects. Since so many studies of the circadian rhythm and its regulation are now being conducted with insects, it seems desirable also to obtain information on metabolic rhythms in insects. To investigate the feasibility of such a study, we decided to examine first the blood sugar concentration, at different times of the day, of the house cricket, which is known to have a marked rhythm of locomotory activity (*1*).

Since the discovery and identification of trehalose in the blood of the silkworm, *Telea polyphemus,* by Wyatt and Kalf (*2*), this disaccharide has been found in many other insects (*3*), where it is frequently the major carbohydrate constituent of the hemolymph. We therefere attempted to identify the blood sugars of cricket hemolymph and to find whether trehalose was a major component in this insect.

The sugars in cricket hemolymph were identified by a combination of paper chromatography (by the method of Gray and Fraenkel, *4*), and paper ionophoresis (by the method of Aso and Hamada, *5*). The results are shown in Table 1, in which R_G and M_G are the observed rates of migration relative to glucose. Two main spots were found, spot I being always greater and more dense than spot II. When eluates of spot I were again subjected to chromatography and tested with Benedicts reagent before and after hydrolysis, their behavior was the same as that of trehalose. From a consideration of its R_G and M_G values, its nonreducing nature, and production of only glucose on hydrolysis, spot I was identified as trehalose. spot II was identified as glucose.

For the quantitative analysis, the method of Somogyi (*6*) and a modified anthrone method (*7*) were used. These methods were simple, gave reproducible results, and allowed for a simultaneous assay of total sugar (anthrone) and reducing substances (Somogyi). We originally attempted to make the blood sugar analyses on single individuals, but the variation between crickets was found to be too great to make this sort of assay practical. Therefore, at least five crickets were bled for each sample of 10 to 100 mg of hemolymph. The samples were taken from crickets which were maintained under regular alternating conditions of light and darkness (LD 12 : 12; light from 8 a.m. to 8 p.m. E.S.T.). Blood samples were taken only once from each batch of crickets which were then not used for any further experiments. Blood from three groups of adult crickets was assayed at eight different times of day. This first series of assays was carried out over a period of about 4 months, since the limited production of crickets allowed only one assay at any particular test hour to be made on any one day. The choosing of any one of the eight times of assay for any particular day was made at random. The mean values for blood sugar concentration obtained from data for at least ten different days, for each of the sampling hours, are plotted in Fig. 1.

There was a definite peak in the concentration of total sugar at 5 a.m. in each group of crickets. No significant corresponding peak in the concentration of reducing substances was found. Though it is realized that the values for reducing substances may also include substances other than glucose, it is suggested that glucose does represent at least a considerable fraction of the reducing substances. It is also suggested that the difference, total sugar minus reducing sugar, is an estimate of the trehalose fraction.

Because of the wide variation and the discrepancies between the individual curves shown in Fig. 1, the blood sugar assays were repeated with a greater number of samples at 5 p.m.

Table 1. Identification of sugars in the hemolymph of the house cricket.

Chromatography, R_G values		Ionophoresis, M_G values	
Av.	Range	Av.	Range
	Blood: Spot I		
0.49	0.42–0.53	0.19*	0.14–0.21
	Blood: Spot II		
0.98	0.93–1.01	1.00	0.97–1.02
	Glucose		
1.00		1.00	
	Trehalose		
0.49	0.38–0.54	0.23	0.19–0.28

* The M_G value for spot I indicates a 1,1 linkage in the disaccharide.

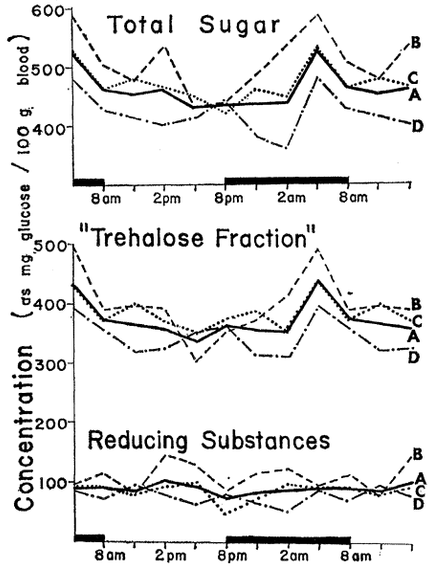

Fig. 1. Daily pattern of blood sugar concentration. Average values for (*A*) all determinations; (*B*) male crickets 14 to 18 days after final moult; (*C*) female crickets 14 to 18 days after final moult; (*D*) males and females 29 to 35 days after final moult. The "trehalose fraction" is the difference, total sugar minus reducing substances.

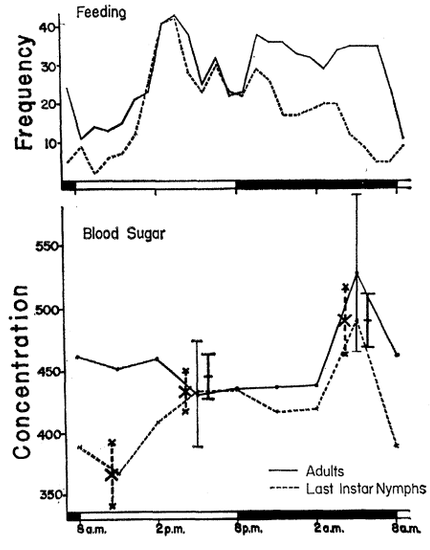

Fig. 2. Daily patterns of feeding and total sugar concentration in the hemolymph. The 95 percent confidence limits are shown for adults at 5 p.m. and 5 a.m. (thin line, first series; heavy line, second series) and for nymphs at 11 a.m., 5 p.m., and 5 a.m.

and 5 a.m. The results of this series and of the first series, shown in Fig. 1, were analyzed according to Student's *t*-test (Table 2). The differences between 5 p.m. and 5 a.m. in the total sugar concentration and the estimated trehalose concentration were highly significant. There was no difference in the values for total blood sugar, at the same time of day, between the first and second series, indicating that there is no significant day to day variation for a sample population of particular age when the crickets are maintained under similar conditions.

The crickets assayed for blood sugar had food available to them right up to the time of assay. Although a daily rhythm of feeding behavior has been observed in the house cricket (*8*), there appears to be no direct relationship between this and the daily fluctuation in the blood sugars. A comparison of feeding behavior and blood sugar concentration is shown in Fig. 2. The patterns of feeding behavior were obtained by observing batches of adults and last-instar nymphs maintained in light and darkness (LD 12 : 12) and counting the feeding frequency during a 10-minute observation period every hour. The frequency counts were made on seven pairs of groups, each of 30 crickets selected at random, on seven different occasions, with each series of observations covering 24 hours.

The concentration of total blood sugar in last-instar nymphs fluctuates just as much as that in adult crickets. This would indicate that the blood sugar concentration is not directly dependent on the locomotory activity, since it has been found (*8*) that last-instar nymphs generally do not show the marked daily rhythm of locomotory activity as do adult crickets.

To confirm the suggestion that the value for total sugar minus reducing substances gives an estimate of the trehalose fraction, which in turn is responsible for the daily fluctuation in the concentration of total sugar, quantitative determinations of the trehalose concentrations were made according to the method of Wyatt and Kalf (*9*). Deproteinized extracts of hemolymph were assayed for total sugar and, after acid and alkaline hydolysis, for trehalose. The results of these determinations are shown in Table 3.

The somewhat lower values obtained for this series of determinations as compared with those for the first two may be explained in part by the use of trehalose in place of glucose as the standard. This would account for about 5 percent of the decrease. Also, there may have been some real change in the blood sugar values since these determinations were made more than 1 year after the first two series. The crickets were maintained on a commercial feed (chick starter mash) which could vary in its composition from year to year and possibly affect the blood sugar levels. However the difference in total sugar between 5 a.m. and 5 p.m. can be accounted for completely by the trehalose fraction. The difference, total sugar minus trehalose, is comparable to the value for reducing substances in the first series and also shows no fluctuation. Hence it is shown that the 5 a.m. peak in total sugar is caused by a peak in the trehalose concentration in the hemolymph.

The finding of a significant daily fluctuation in the trehalose concentration of cricket hemolymph suggests that variations in the concentrations of other metabolites should be studied in insects. Information on such physiological fluctuations could be useful in reaching an understanding of circadian organization.

Table 2. Concentration of total sugar and reducing substances (RS) in the hemolymph of crickets at 5 p.m. and 5 a.m., and the difference, total sugar minus reducing substances (expressed as micrograms of glucose per 100 mg of blood).

Substances	Time	No. of samples	Blood sugar concentration			Significance of difference
			Av.	95% Confidence limit	Difference	Calculated *t*
First series: blood samples from adult crickets						
1) Total sugar	5 p.m.	18	431	42		
	5 a.m.	17	527	61	96	2.74*
2) RS	5 p.m.	18	94	27	3	No significant difference
	5 a.m.	17	91	17		
3) Difference	5 p.m.	18	337	38		
	5 a.m.	17	436	49	99	3.38*
Second series: blood samples from adult crickets						
1) Total sugar	5 p.m.	47	446	18		
	5 a.m.	48	490	20	44	3.19*
Second series: blood samples from last-instar nymphs						
1) Total sugar	5 p.m.	24	434	16		
	5 a.m.	30	489	27	55	3.38*

* The calculated *t* values indicate significance at the 99 percent level.

Table 3. Concentration of total sugar and trehalose in the hemolymph of adult crickets at 5 p.m. and 5 a.m., and the difference, total sugar minus trehalose (expressed as micrograms of trehalose per 100 mg blood).

Substance	Time	No. of samples	Blood sugar concentration			Significance of difference
			Av.	95% Confidence limit	Difference	Calculated
1) Total sugar	5 p.m.	30	358	31		
	5 a.m.	32	414	22	56	3.04*
2) Trehalose	5 p.m.	30	281	29		
	5 a.m.	32	330	18	49	2.96*
3) Difference	5 p.m.	30	79	22		No significant difference
	5 a.m.	32	84	14	5	

* The calculated *t* values indicate significance at the 99 percent level.

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References and Notes

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