Glucose Levels as Caste Indicator of the Honeybee (*Apis mellifica ligustica*)

Caste determination of the honeybee is a problem as old as the domestication of the insect. The subject was reviewed by REMBOLD and HANSER[1], REMBOLD[2], WILSON[3], and by SHUEL and DIXON[4], who claimed that preferential feeding of the larvae and/or the composition of their diet is crucial for the development of the different castes. On the other hand, FISCHL and ISHAY[5], in an investigation of the Oriental Hornet (*Vespa orientalis*), found that significant differences in glucose levels exist in the hemolymph of the castes with no relationship whatsoever to diet. Furthermore, it was found that upon incubation of the hemolymph with endogenic hyperglycemic factors (components present in the hemolymph and to a greater extent in the midgut), different glucose curves were produced by the various castes[6].

In the present study, measurement is made of the glucose levels in the hemolymph of different castes of the honeybee, and the changes that occur in those levels after incubation of the hemolymph alone and with midgut. The interspecies effect with material obtained from hornets is also investigated.

Materials and methods. *Apis mellifica ligustica* larvae were used in the experiments, the queen and worker larvae layed in the same hive by one queen on one day. On precisely the 8th day they were starved for 12 h, and immediately removed from their cells. To remove residual food and debris, they were washed in distilled water and gently dried with filter paper. The larvae were punctured in the dorsum near the heart with a sterile needle and the hemolymph from each group (queen and worker) was pooled into ice-cooled test tubes. Midgut was removed separately and its open side firmly tied with silk thread. Midgut and hemolymph were obtained in the same manner from *Vespa orientalis* larvae.

The pH of the samples was set to 7.0 and the incubation was continued for 90 min at 37 °C. Incubation with midgut was done by immersing the intact midgut into the hemolymph. Glucose blank of the midgut was obtained by incubating it immersed in Ringer's solution.

Hydrolysis was performed with 0.1 *N* HCl in sealed ampules, heated in boiling water bath for 3 h. Glucose determination was made according to the enzymatic method of KINGSLEY and GETCHEL[7]. All experiments were performed three times.

Results. Glucose levels of the honeybee hemolymph rose steadily upon incubation, with significant differences between the castes, both in the initial value and in the rates of increase (Table I). As summarized in Table II, the honeybee midgut contained considerable amounts of glucose, while the levels in the hornet midgut are low. Incubation of midgut immersed in the hemolymph produced a more rapid and greater increase in the glucose levels than hemolymph incubated alone, and the values found were significantly higher than the sum of the hemolymph and midgut incubated separately (Table III). The castes differed significantly both in the glucose levels and the ratio of glucose production pre- and post-incubation (Table IV).

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Table I. Increase of glucose levels in the hemolymph of the honeybee on incubation and after acid hydrolysis

Time of incubation (min)	Worker larvae			Queen larvae		
	Glucose (mg/100 ml)	Rate (%)	Hydrolysis gluc. (mg/100 ml)	Glucose (mg/100 ml)	Rate (%)	Hydrolysis gluc. (mg/100 ml)
0[a]	605	0	3200	1870	0	2150
30	800	32		2105	12	
60	935	55		2220	19	
90	1165	93	4000	2450	31	2500

Table II. Perfusion of glucose into Ringer's solution from the midgut of *Apis mellifera* and of *Vespa orientalis*, and glucose values after hydrolysis

Time of incubation (min)	*Apis mellifica ligustica*						*Vespa orientalis*	
	Worker larvae			Queen larvae			Glucose (mg/100 ml)	Hydrolysis gluc. (mg/100 ml)
	Glucose (mg/100 ml)	Rate (%)	Hydrolysis gluc. (mg/100 ml)	Glucose (mg/100 ml)	Rate (%)	Hydrolysis gluc. (mg/100 ml)		
0[a]	2005	0	3200	420	0	720	15	17
30	2640	32		680	62		17	
60	3085	54		720	71		20	
90	3762	81	3900	780	86	800	30	40

Table III. Gluconeogenesis induced by midgut on the hemolymph of honeybee and of the hornet

Hemolymph	AW		AW		AQ		V		V		AQ		V	
Midgut	AW		V		AW		V		AW		AQ		AQ	
Time of incubation (min)	G	R	G	R	G	R	G	R	G	R	G	R	G	R
0[a]	2605	0	2605	0	3900	0	115	0	2189	0	2225	0	540	0
30	3100	19	3860	48	3100	−21	140	22	3380	55	2900	30	1100	104
60	4700	80	6770	160	2700	−26	160	39	4400	101	3085	39	1400	160
90	4700	80	8250	217	2500	−36	210	82	5100	133	3085	39	1500	180
Hydrolysis	8500		8450		4000		350		5100		3300		1500	

AW, Honeybee workers; AQ, honeybee queens; V, hornet; G, glucose (mg/100 ml); R, rate of glucose change (%). [a] see Table II.

Table IV. Key for the biochemical caste differentiation of the honeybee

	Initial glucose levels of the hemolymph	Initial glucose levels of the midgut	Hemolymph polysaccharide reserves	Hemolymph glucose generation	Hemolymph midgut glucose generation
Worker larvae	Lower	Higher	Higher	Faster	More active
Queen larvae	Higher	Lower	Lower	Slower	Less active

Discussion. As illustrated in Table I, the average glucose level in larval hemolymph of the worker honeybee is only about a third that of the queens. However, the rate of increase on incubation is much higher in the workers. With acid hydrolysis, the glucose levels of the workers are much higher both in the pre- and post-incubation samples ($p < 0.0005$). It is concluded that queens have much more immediately available glucose energy supply than workers, but the latter are capable of mobilising energy at a much higher rate and, when necessary, to a much higher level. It is also noteworthy that acid hydrolysis of both queens' and workers' hemolymph yields more glucose after incubation of the sample than before, possibly due to the presence of endogenous gluconeogenetic factors in the hemolymph.

In previous experiments, we found that the midgut of hornet larvae contains practically no or very little glucose[8], although perfusion with hemolymph greatly accelerates the gluconeogenesis of these larvae[6].

The blank tests on the honeybee midgut immersed in Ringer's solution showed that it differs greatly from the hornet larvae in that the worker's honeybee midgut was extremely rich in glucose and the queen's only somewhat less rich (Table III).

Acid hydrolysis of the homogenized midgut material also have low glucose values in the hornet's midgut. The high 'glucose-blank' of the honeybee midgut somewhat complicates the interpretation of the results, but even so it is clear that the total glucose generated is higher than the sum of the hemolymph and the midgut incubated separately (Table III). Fortunately, it was found that an interspecies relationship exists and the midgut of each species and any caste will act similarly on hemolymph of either source. This accounts for the results obtained with hornet midgut with which the assessment of the gluconeogenesis is much more clear.

It is also interesting to note (Table III) that when the larval hemolymph of worker bees and hornets was incubated with its own midgut, the rate of the glucose production was lower than when interspecies material was used. On the other hand, the midgut of worker bee larvae was not able to induce gluconeogenesis in queens' hemolymph. We cannot offer an explanation for this phenomenon without further study.

Table IV presents a simplified interpretation of the results for practical application in caste determination. It must be noted that this table gives only the general trends of the glucose ratios of the castes instead of the absolute figures. This is because the seasonal, nutritional and developmental differences of glucose levels in the same castes may be much more greater and more variable than the intercastal variation, making the numerical data unsuitable for caste determination.

Résumé. L'hémolymphe de l'abeille devient hyperglycémique pendant l'incubation en présence de l'intestin moyen prélevé sur larves d'abeilles ou de guêpes. Des différences significatives ont été observées entre les larves de l'abeille ouvrière et celles de la reine tant en ce qui concerne le taux du glucose dans l'hémolymphe que celui de son augmentation après l'incubation en présence des différents facteurs hyperglycémiques tels que l'intestin moyen des larves. Cela suggère que ces facteurs doivent être considérés comme caractéristiques des castes chez les abeilles.

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