Table 1. *Neuronal groups and their cell numbers in the cervico-thoracic ganglia of the larva of G. mellonella.*

Nerves filled	Neuronal groups				Cell numbers+
	SOG	T_1	T_2	T_3	
cn	1	–	–	–	3
mn-1	–	2,3	–	–	*1,2
mn-2	–	–	2,3	–	2,2
mn-3	–	–	–	2,3	2,2
dn-1	4–8	9–11	–	–	1,1,1,9,1 & 2,1,2
dn-2	–	4–8	9–12	–	1,1,1,9,1 & 2,1,2,4*
dn-3	–	–	4–8	9–12	1,1,1,9,1 & 2,1,2,2

+ each count is an average of 10 fillings
* indicate difference in cell numbers in homologous groups

observed in the ganglia is fewer compared to the number of targets their nerves innervate. This should attribute multiple functions to their neurons. No neuronal group or cells specific to the innervation of the PTG was found which further supports our concept of multiple function for these cells.

We are thankful to Dr F. Sehnal of the Institute of Entomology, Prague, Czechoslovakia, for critical evaluation of the manuscript.

1 June 1985; Revised 5 August 1985

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ON THE HYPERGLYCEMIC HORMONE OF THE FRESHWATER CRAB, *BARYTELPHUSA GUERINI* MILNE EDWARDS

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THE hyperglycemic hormone (HGH), regulating the blood sugar level in crustaceans, is separated and its properties are elucidated leading to its substantial purification[1–8]. Separation, characterisation and purification of eyestalk principles such as HGH are important and need greater emphasis, since such studies may clarify a number of possibly related physiological effects[3].

The hyperglycemic hormone is found in the eyestalks of the crab, *Barytelphusa guerini*[9]. A preliminary report on separation and purification of this hormone is made in this paper. Collection, maintenance and choice of animals for experimentation as well as bilateral eyestalk ablation were described earlier[10].

The eyestalks were triturated into a fine paste in a glass homogenizer and suspended in distilled water to make a final concentration of one pair of eyestalks/0.2 ml. After centrifugation, the supernatants were divided into two portions: one constituting the unboiled extract was directly used for injection purposes and the second portion was heated at 100°C for 20 min. The final volume after boiling was adjusted to the original volume by adding distilled water. It was centrifuged for 10 min at 3000 rpm and the clear supernatant constituted the boiled extract. The eyestalks were either frozen in a freeze chamber or air-dried at room temperature for 24 hr and were used for preparing frozen and air-dried eyestalk extracts respectively.

An extract of the required number of eyestalks was prepared using acetone, and it was centrifuged at 3000 rpm for 10 min. The supernatant and residual fractions were taken in two test tubes and allowed to remain at 26–28°C for acetone evaporation. The dried acetone soluble and insoluble fractions were dissolved in suitable amounts of distilled water and again centrifuged. The supernatants were separated and made upto a final volume to make one pair of

eyestalks 0.2 ml. These constituted the acetone-soluble and acetone-insoluble extracts of the eyestalks.

Alcohol soluble and insoluble fractions of the eyestalk extracts were also prepared in a similar manner. The extract (0.2 ml) containing a pair of eyestalks was injected into normal animals with intact eyestalks and eyestalk-ablated animals and the blood sugar level was estimated 1 hr after injections, using Anthrone method[11]. Blood sugar levels were also estimated in the normal and eyestalkless animals injected with 0.2 ml of freshwater crab ringer[12] for the purpose of comparison.

The blood sugar levels in the normal and eyestalk-ablated animals showed nearly 400 and 300 % increase respectively, after the injection of the unboiled and boiled aqueous eyestalk extracts. The extracts of eyestalks subjected to different treatments such as boiling, freezing and air drying also caused high hyperglycemia comparable with that caused by the unboiled extracts (figure 1A & 1B). The hyperglycemic level caused was also significant ($P < 0.01$ at 5 % level) when compared with control animals.

Acetone and alcohol insoluble fractions of eyestalk extracts caused a 400 % increase in the blood sugar level of normal and eyestalk-ablated animals. This hyperglycemic level was equivalent to that produced by the normal aqueous eyestalk extracts, and was significant ($P < 0.01$ at 5 % level), when compared with control animals. On the other hand, the alcohol and acetone soluble fractions of the eyestalk extracts did not alter much, the glycemic level in both normal and destalked animals (figure 2A & 2B).

Interestingly, the hyperglycemic response produced by the different eyestalk extracts is more in the normal animals than in the eyestalk-ablated ones. The normal individuals maintain normal blood sugar levels due to the availability of HGH in the eyestalks and greater response in these animals is due to the additional titre of HGH received through extract injections. On the other hand, the ablated animals are already in hypoglycemic condition due to the non-availability of HGH on account of eyestalk removal and hence the extract injections, though capable of increasing the blood sugar level, do so to a small extent. Thus the response of the animals to the injected HGH seems to depend upon the initial availability of HGH in the recipients and their glycemic level.

The results suggest that the hyperglycemic factor in *B. guerini* is thermostable as found in Uca sp.[13], *Scylla serrata*[14] and *Parapenaeopsis hardwickii*[15] but differs from the HGHs from *Pandalus borealis*[6], *Orconectes limosus*[16], *Cambarus robustus*[17], *Varuna*

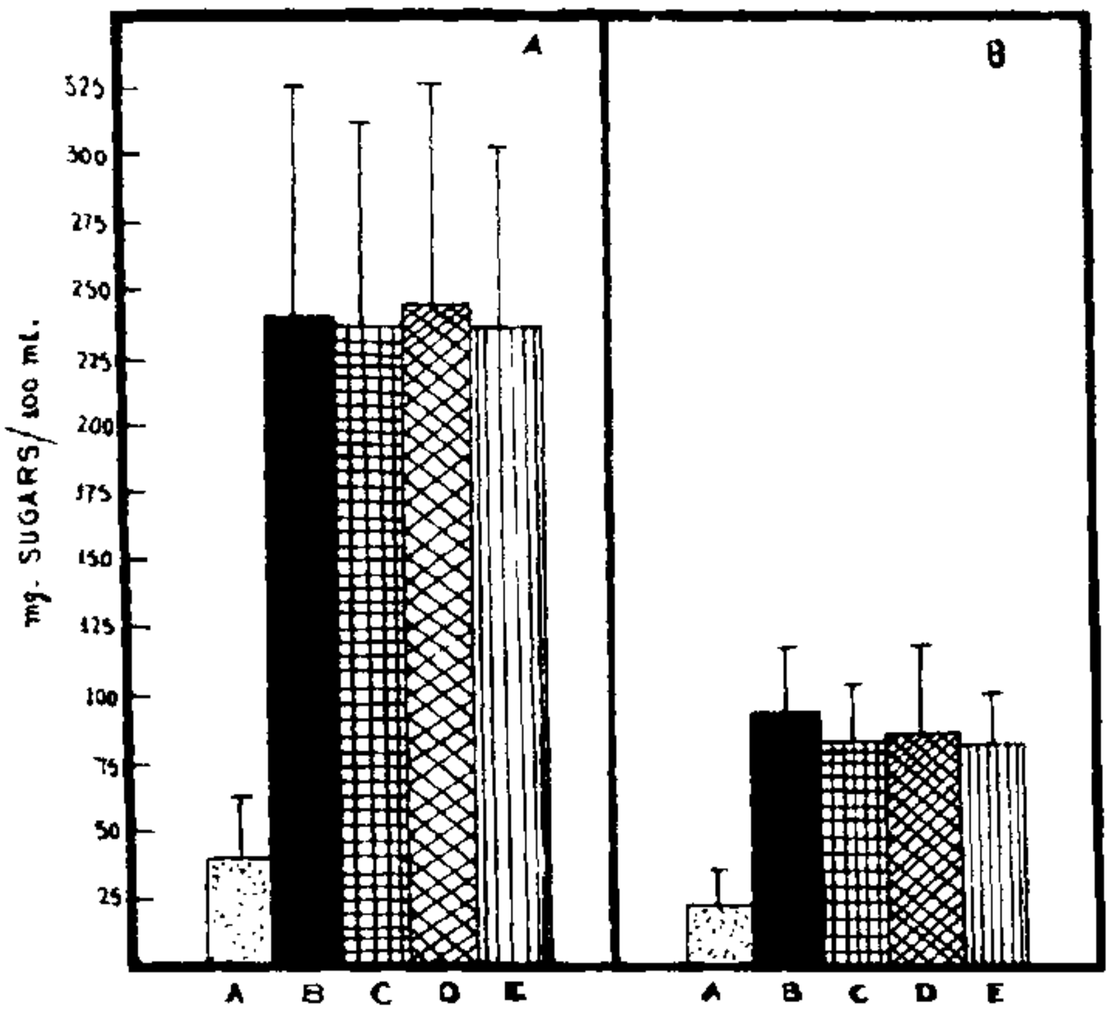

Figure 1. Blood sugar level in the intact (A) and eyestalk-ablated (B) crabs on injection of eyestalk extracts subjected to different treatments. Values are the averages of 6 observations ± S.D. A—control, B—unboiled, C—boiled, D—frozen, E—air-dried.

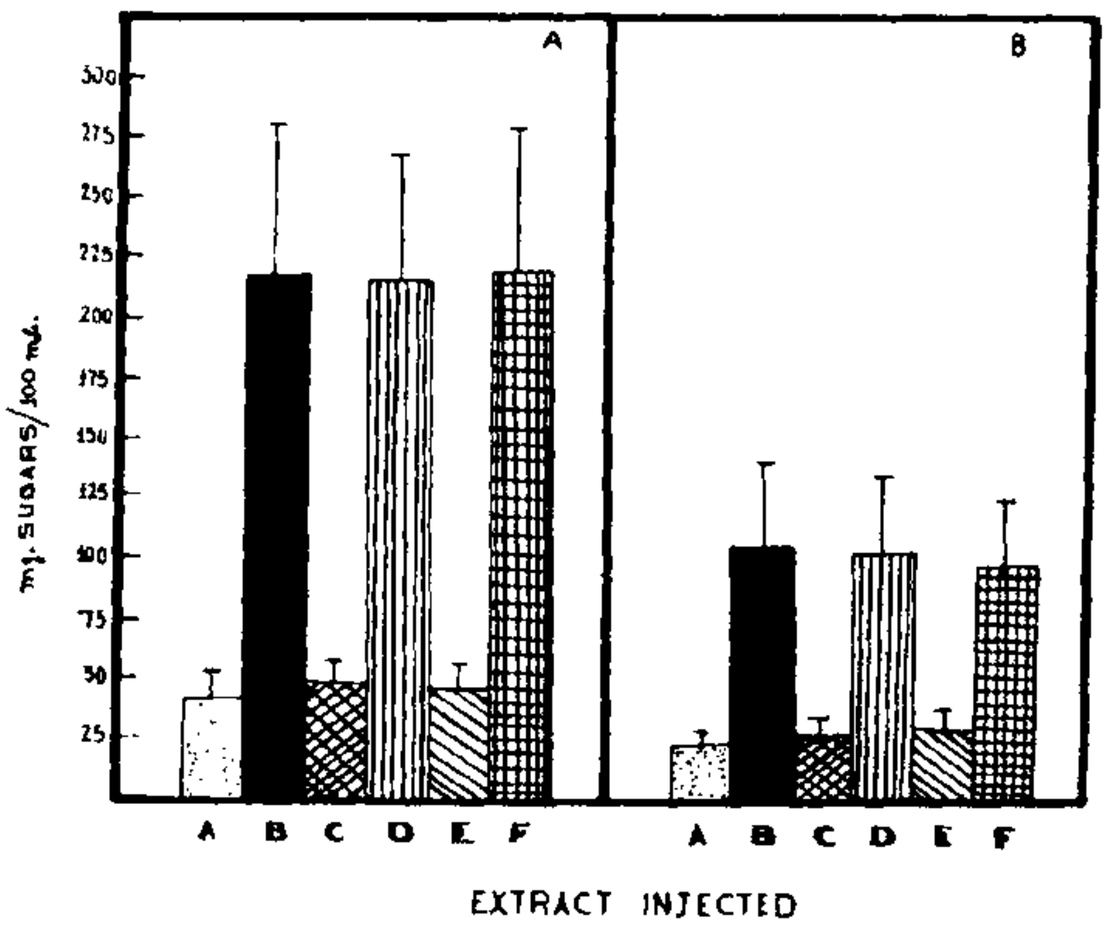

Figure 2. Blood sugar level in the intact (A) and eyestalk-ablated (B crabs on injection of different fractions of eyestalk extracts. Values are the averages of 6 observation ± S.D. A—control, B—aqueous, C—acetone soluble, D—acetone insoluble, E—alcohol soluble, F—alcohol insoluble

litterata[18], *Panulirus polyphagus*[19], which are thermolabile. Because of this high thermostability even the boiled extracts could bring about pronounced hyperglycemia to the same extent as the unboiled extracts.

The hyperglycemic factor seems to be resistant to freezing and desiccation also, in addition to heating and hence the hyperglycemic effect is very well produced on injection of these extracts into both the normal and eyestalk-ablated animals.

Separation of hormones using the fractionation technique suggests that the hyperglycemic hormone is insoluble in both acetone and alcohol. Further the hyperglycemic effect produced by the insoluble fractions is as much as that produced by the aqueous eyestalk extracts in both the normal and eyestalkless animals suggesting that there is no loss of activity due to fractionation. The hyperglycemic hormone in *Pandalus borealis*[6] and *Orconectes limosus*[16] is acetone soluble and can be precipitated by treatment with saturated ammonium sulphate solution.

Further, the chromatographic and electrophoretic analysis shows that it is a protein molecule of relatively smaller molecular size[3,6,16,20]. HGH, from *Orconectes limosus*[7], *Carcinus maenas*[8] and *Cancer magister*[21], contains fewer number of amino acids, as evident from the amino acid composition.

The highly thermostable nature of the hyperglycemic hormone in *Barytelphusa guerini* and its resistance to desiccation and freezing, possibly suggest that it does not get easily denatured or lose its activity and hence may not be a protein. Its high solubility in aqueous media and insolubility in lipid solvents such as acetone and alcohol, precludes the possibility that it is a lipid material. It may be tentatively argued that this hormone is a polypeptide. Further steps in the characterisation of this hormone such as digestibility by proteolytic enzymes; dialysability, electrophoretic mobility etc would be required for confirming this view and investigations along these lines are in progress.

Partial support from UGC, New Delhi by way of National Research Associateship to one of the authors (MSG) is gratefully acknowledged. MSG also wishes to thank Principal K. H. Shitole and Prof. S. K. Aher for constant encouragement.

17 November 1984; Revised 2 September 1985

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A TECHNIQUE FOR IDENTIFICATION OF THE INSTAR OF *MANSONIA* LARVAE

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THE present paper deals with a new technique developed in this laboratory to identify different instars of *Mansonia annulifera* and *Ma. uniformis* based on the details of ornamentation and architecture of the respective respiratory siphon using exuviae.

The biology of *Ma. richiardii* and *Ma. uniformis* was studied in detail by Laurence[1]; however, only a