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.

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

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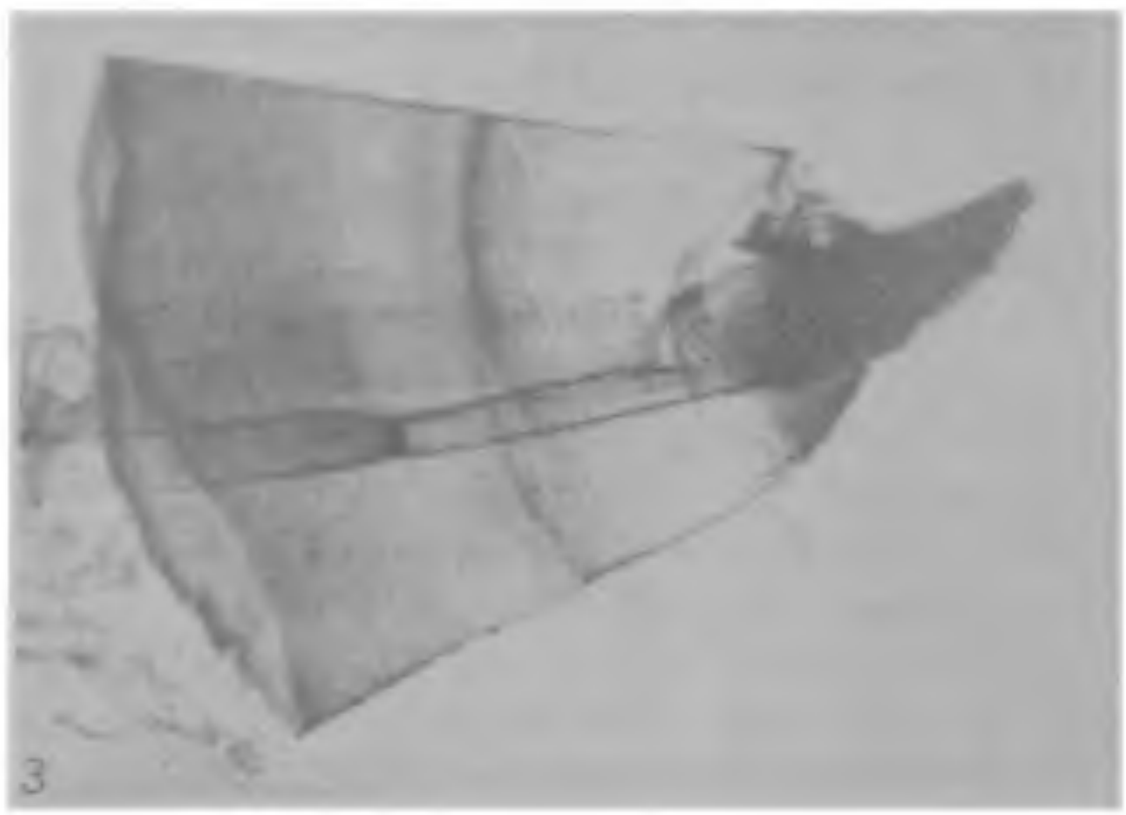
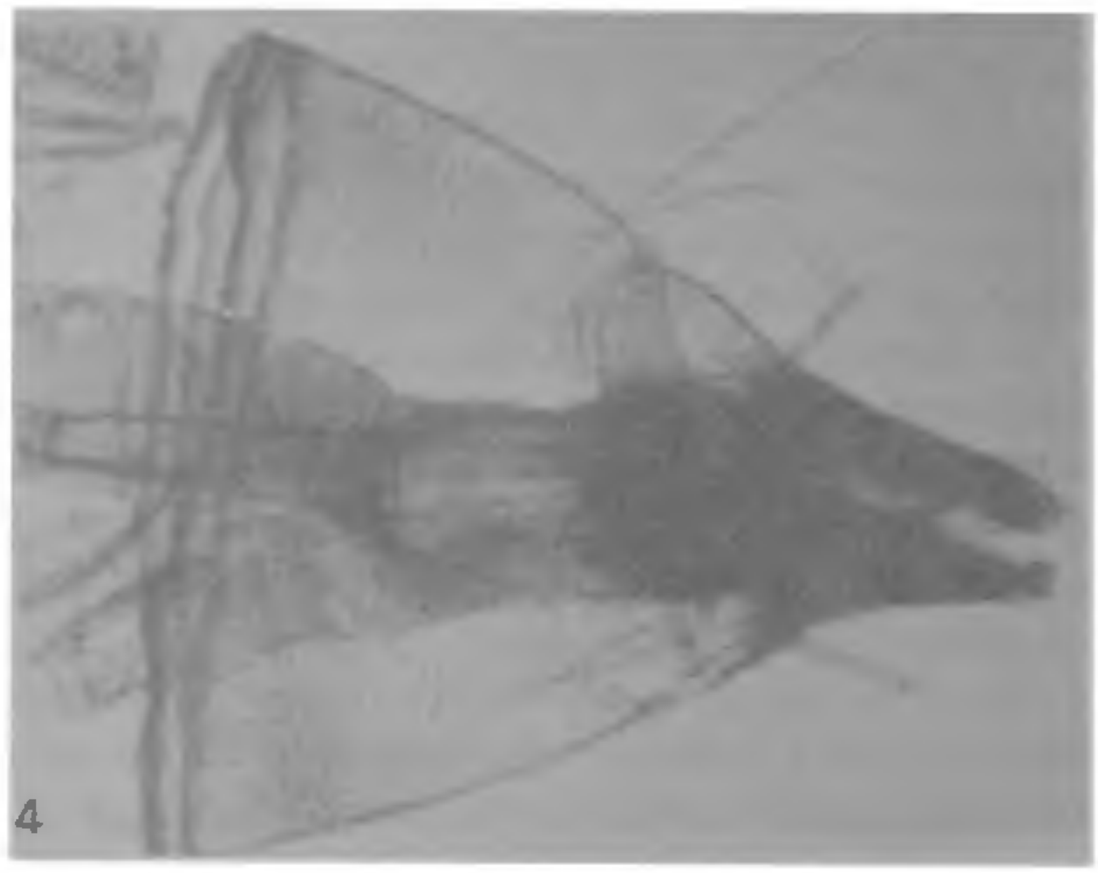
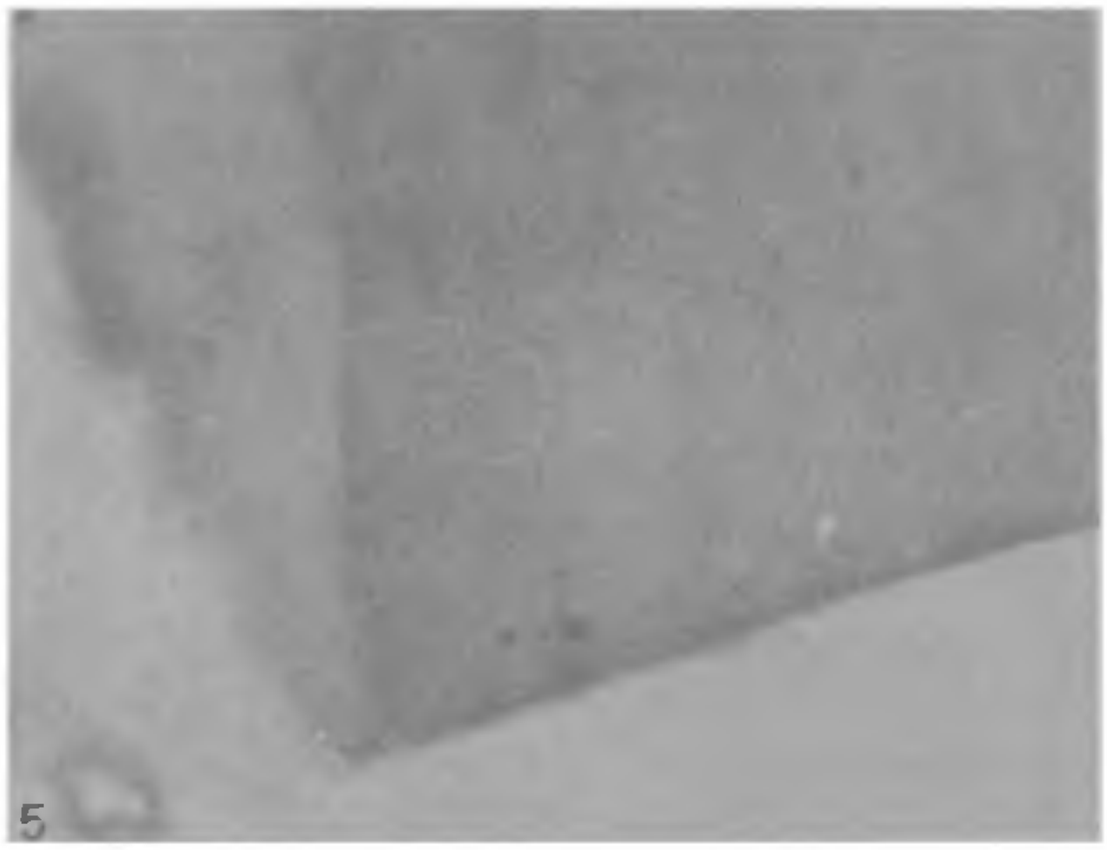
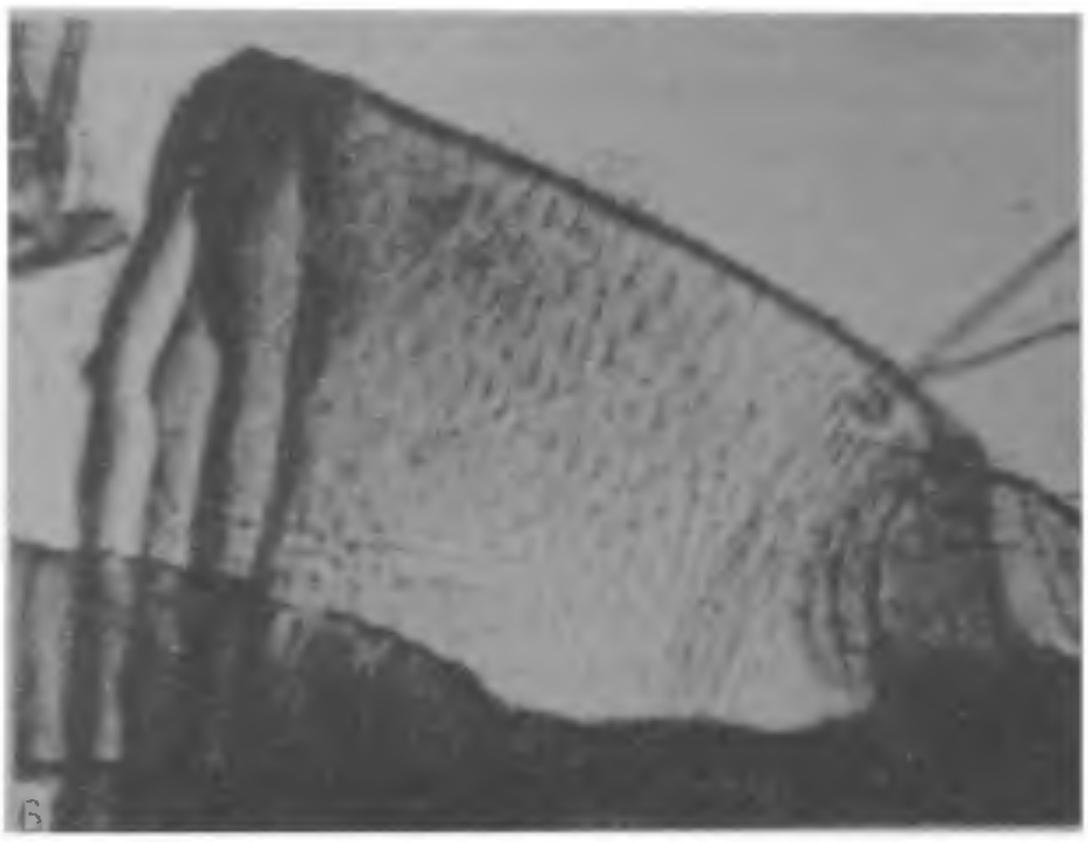
THE present paper deals with a new technique developed in this laboratory to identify different instars of *Mansonia annulifera* and *Ma. uniformis* based on the details of ornamentation and architecture of the respective respiratory siphon using exuviae.

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









Figures 1–6. Larval respiratory siphon of *Mansonia uniformis*; **1–4** ca × 650, **5–6** ca × 850. **1.** First instar—note the absence of cuticular ornamentations. **2.** Second instar—Base of the siphon is dark brown in colour (note the ridges and furrows) and the top is less dark brown in *colour with dentate* ornamentation. **3.** Third instar, **4.** Fourth instar—Note the restriction of the cuticular ridges and furrows to a double ring like structure at the base of the siphon and the even distribution of the dentate ornamentation at the top. **5.** A portion of figure 3 enlarged exhibiting the pattern of cuticular ridges and furrows, **6.** A portion of figure 4 enlarged, showing the pattern of distribution of the cuticular dentate ornamentation.

mention was made regarding various instars of the larvae. On the other hand, Wesenberg-Lund[2] compared, in general, the difference in structure of the saw-like median plates of the siphon of the first instar larvae with the siphon of the other instars. This, perhaps, is useful only to differentiate the first instar larvae from the rest of the instars. The present study reveals that cuticular architecture and coloration are very promising and easily recognisable characters to differentiate various instars of *Mansonia* mosquitoes.

The whole mounts of both larvae and exuviae were prepared in polyvinyl alcohol and they were subjected to study to elucidate the cuticular pattern of the siphon.

There is a remarkable difference in the size and shape of the respiratory siphon of the first instar larva in comparison with the other 3 instars (figures 1–4). In the former, the toothed region of the piercing stylet is confined to the tip (figure 1) whereas in the latter case it runs along the whole length of the stylet as reported by Burton[3]. This facilitates the larva piercing into thicker roots. The shape of the siphon is almost similar from the second instar onwards, though their sizes are different. If a ratio between the length and breadth (measurement at the base which is the broadest) is taken, it shows a tendency towards reaching unity. The ratio is 0.44 (2/4.5 cm) for the first instar while for the rest of the instars the ratios are 0.62 (3.2/5.2 cm); 0.75 (5.5/7.5 cm) and 0.89 (6.5/7.3 cm) for the second, third and fourth instars respectively (all micrographs were taken at the same magnification, except figures 5 and 6, and the measurements were taken directly from the photoprints).

The breadth of the siphon increases as the larvae advance in development (cf figure 1 with figures 2,3 4). There is very little increase in length after the larva has moulted into the third stage, but the breadth increases. This results in the ratio approaching unity in the 4th instar larva.

Another characteristic change that takes place, as the larva grows, is with the cuticular architecture of the siphon (figures 2,3,4,5 and 6). These variations give definite clue to the exact stage of the larva. The basal half of the siphon of the 2nd and the 3rd instar (figures 2 and 3) is more dark brown in colour than the other regions. This dark brown region shows lines of ridges and furrows (figure 5) as seen in human palm whereas the light distal region of the siphon shows dentate ornamentation (figure 6); this becomes more prominent in the third instar larvae. A consistent feature of the third instar larva is the appearance of a dark brown ring which separates the two regions mentioned above. In the fourth instar larva (figure 4) the dark brown region disappears and is represented only by thin dark brown rings at the base of the siphon. The dentate ornamentations which are confined to the distal half of the second and third instar larvae, now occupy the whole portion of the cuticular area of the siphon except the dark brown region at the base which still has the ridges and furrows, a reminiscent of the second and third instars (figure 4).

In addition to the ornamentation of the siphon, as with the growth of the larvae, a change in number, size and shape of the dorsal tuft and the ventral brushes of anal segment was also noticed. Increase in number and the spreading of the hairs from their origin to the distal region progressively changed from first to the fourth stage.

The greatest advantage of the study is that these cuticular changes can be easily studied from various larval exuviae at different stages.

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