Gel Filtration of the Hemolymph of the Honey Bee (*Apis mellifera* L.)

Adult worker honey bees (*Apis mellifera* L.) produced agglutinating substances in response to an injection of a vaccine prepared from *Bacillus larvae* White¹. Since these substances are expected to be protein, one step in their clarification is an investigation of the proteins in bee hemolymph. Separation of hemolymph protein by polyacrylamide gel disc electrophoresis recently showed that the hemolymph of adult worker honey bees contains 9 protein bands which remain unchanged in the adult regardless of the age or diet of the insect².

The present investigation was therefore made at the Tucson Bee Research Laboratory in 1968 to estimate the molecular weights of the proteins in honey bee hemolymph by gel filtration on Sephadex G-200 with rabbit hemolysin as a standard. I selected rabbit hemolysin as the mammalian serum because rabbit antibody to sheep erythrocytes has a sedimentation constant of 18 to 19^{3,4}. Thus, hemolysin contains all types of serum globulins and albumin.

Sephadex[®] is a modified dextran which separates components of a mixture on the basis of their molecular size. Sephadex G-200 has been used to fractionate mammalian serum proteins⁶ and has a fractionation range of 5,000 to 800,000 mol weight (Mw) for peptides and globular proteins⁷. The largest globulin, IgM (Mw $\approx$ 900,000)⁸, appears in the effluent as the first component. Other smaller globulins follow, including IgG (γ -globulin), which has a Mw of about 160,000⁹. Albumin, which has the lowest Mw (68,000) of the major serum components¹⁰, is the last component to leave the column.

Hemolymph of adult worker honey bees was collected from the thorax as previously described¹. Rabbit antiserum against sheep erythrocytes (hemolysin) was prepared by the method of CAMPBELL et al.¹¹ using the i.v. route; the titer of this serum was 4000.

A 2 $\times$ 20-cm glass chromatographic column was packed with Sephadex G-200 supported by glass wool and a thin layer of Sephadex G-25. Swelling of the Sephadex G-200 beads was accomplished with 0.1M tris-HCl buffer, pH 8.0, and 0.2M NaCl for 3 days. Then 1 ml of the hemolymph or

hemolysin was layered on the column for fractionation. The separation was carried out at 22°C using the buffer and 0.2M NaCl with a flow rate of 10–20 ml/h. The fraction volume was 5 ml. The protein concentration was estimated by measuring the optical density (OD) of the samples at 280 nm.

The figure shows the results of the fractionation of hemolysin and honey bee hemolymph. Rabbit hemolysin contained a large amount of 19S IgM (1st peak), a 7S IgG peak (2nd peak), and a 3rd peak containing albumin. The 19S hemolysin peak again gave a titer of 4000. No other peaks contained antibody to sheep erythrocytes. Honey bee hemolymph contained 2 large fractions, a peak in the 19S region and another peak between the 19S and 7S region of hemolysin.

Thus, bee hemolymph contains a large fraction which has a Mw similar to that of 19S IgM in hemolysin. A 2nd peak of unknown Mw was found between the 7S and 19S fractions of hemolysin, and a 3rd slight peak (tubes 24–30) corresponded to the 7S region. A slight rise in OD was also noted in the albumin region.

It is not yet known which protein bands separated by polyacrylamide gel disc electrophoresis² correspond to the peaks obtained by Sephadex gel filtration. However, we now have some idea of the Mw of the proteins in honey bee hemolymph.

Zusammenfassung. Proteine von Bienenhaemolymph und Kaninchenhaemolysin wurden nach Trennung an Sephadex G-200 verglichen. Aufgrund der bekannten Molekulargewichte der Haemolysineproteine konnten Anhaltspunkte für das Molekulargewicht der Haemolymphproteine gefunden werden.

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Fractionation of hemolysin and honey bee hemolymph on Sephadex G-200.

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