

A Mouse Toxic Hemagglutinating Factor in Keyhole Limpet Hemolymph

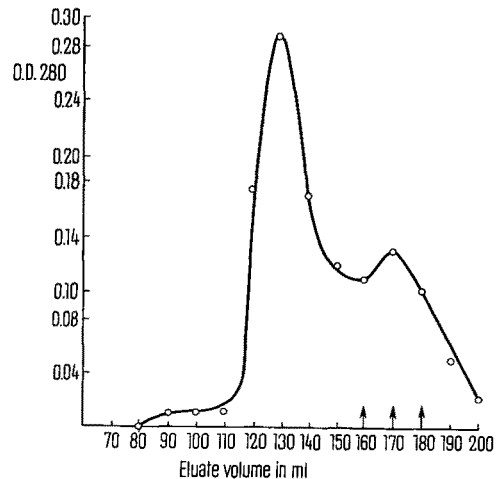
Large quantities of hemolymph can be obtained from the American keyhole limpet (*Megathura crenulata*) by incising the mantle close to the shell and allowing the blueish fluid to drain out in the cold for several hours. The hemocyanin can be removed from this by centrifugation at 100,000 *g* for 2 h, leaving a clear, colourless supernatant fluid. During a survey of marine invertebrates for the occurrence of factors which agglutinate mammalian red cells, this fluid was found to be highly active. It would agglutinate 1% suspensions of all red cells kept at 0°C during testing to a dilution of approximately 1/2000. The species tested included sheep, human groups A and B, Swiss white outbred mice, Balb/C and C₃H mice and rabbits. If the cells were incubated at 37°C with the hemolymph dilutions then hemolysis resulted within 10 min to the same titre. This activity of the hemolymph was extremely heat labile, 99% inactivation occurring after 1 min at 56° or 10 min at 42°C.

The active fraction was precipitated by 40% saturated ammonium sulphate and could be entirely recovered in the 0.15 *M* saline solution of this precipitate. Further fractionation of this material was achieved by passing 25 mg through Sephadex G200, 2 peaks were obtained and the biological activity was restricted to the later one (see Figure). The tubes 16–18 containing the hemagglutinin were pooled and concentrated to yield 4.8 mg protein containing approximately half of the original activity, giving about 2-fold purification in terms of activity/mg of protein.

This crudely purified material and the original hemolymph were found to possess an extraordinary toxicity for mice following i.v. injection. As little as 0.1 ml of a 1/50 dilution of the original fluid represented an LD₅₀ dose on i.v. injection. Within 30 sec of the injection the mice showed signs of rapid shallow breathing with cyanosis. This was accompanied by other signs of smooth muscle contraction such as emptying of the bladder and defecation. Death occurred within 1 or 2 min, otherwise the mice recovered completely. The LD₅₀ dose of the crude hemolymph represented 10 µg of protein and the material purified on sephadex was about twice as active.

The similarity between the toxic signs seen and those of acute anaphylaxis was striking, this coupled with the high hemagglutinating or hemolytic activity of the material for

mouse cells suggest an intriguing analogy with mammalian antibody systems¹.



Sephadex G200 fractionation of Keyhole limpet hemagglutinin. Column (60 × 2 cm) was eluted with 0.15 *M* phosphate buffered saline pH 7.2. All the activity was recovered in the eluate between 160–180 ml.

Zusammenfassung. Die Hämolymphe der Tellermuschel, *Megathura crenulata*, ist hochtoxisch für die Maus. Gegen Erythrozyten verschiedener Säuger wirkt sie hämagglutinierend und hämolysierend.

M. P. ROWLEY and D. ROWLEY

Department of Microbiology, University of Adelaide, (South Australia), 26 March 1968.

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Proteolytische Aktivität normaler und antigenstimulierter Makrophagen

Ein Kernproblem der modernen Immunologie ist der Locus der Antikörperbildung. Nach den Arbeiten von FAGREUS¹, insbesondere aber nach jenen von WELLER und COONS², wurde festgestellt, dass in den Plasmazellen (Plasmazyten) Antikörper produziert werden. Die Untersuchungen von DIXON³ und später von FISHMAN et al.^{4,5} ergaben, dass am Prozess der Antikörperproduktion auch Mikrophen teilnehmen. Ihre Rolle ist jedoch bisher noch nicht ganz geklärt worden^{6–11}. Dennoch geht aus zahlreichen Befunden hervor, dass als erstes Ergebnis der Wechselwirkung zwischen Antigen und Makrophag RNS produziert wird, die die Antikörpersynthese in den Plasmazyten induziert.

In dieser Mitteilung werden die Resultate der Veränderungen der proteolytischen Aktivität der polymorphen Kernleukozyten nach Einwirkung des Aujeszky-Virus erörtert.

Material und Methoden. Die Untersuchungen wurden auf Polynuclearleukozyten von Meerschweinchen mit

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