

ENTOMOLOGY

Effect of Scorpion Venom on Specific Gravity of Locust Haemolymph

It is known that blood density may be reduced by treatment with many biologically active materials. Patton observed¹ a rise in the specific gravity of the larval haemolymph of *Galleria mellonella* following injection of LD_{50} doses of sodium arsenate. The rise in haemolymph specific gravity was observed in individuals which survived up to 3 h; in organisms surviving thereafter a gradual descent to normal values occurred.

During studies of immunization of locusts (*Locusta migratoria migratorioides* R. and F.) with scorpion (*Leiurus quinquestratus* H. and E.) venom, it was noted that the haemolymph extracted from immunized locusts exhibited certain unique physical properties. In such haemolymph, collected for serological tests in capillary tubes and stored at 4° C, particles were noted after 12 h; after 24 h the whole haemolymph extract became opaque, with considerable amounts of particulate sediment. Haemolymph from untreated locusts, kept under similar conditions, remained clear and only occasionally showed a cloudy precipitate. It was therefore decided to determine whether the physical effects detected in treated haemolymph are associated with changes in its specific gravity.

Haemolymph was collected from treated and control locusts by an adaptation of the technique used by Siakotos² for cockroaches. This involved removal of the posterior end of the abdomen in the region of the 7th segment, decapitation, and removal of the alimentary canal by tearing the cervical membrane. The hollowed bodies were gently centrifuged (300 r.p.m., 2-3 min) in special tubes in which the haemolymph of 4 locusts from the same batch were pooled. Treated locusts and controls were of identical age, sex and breeding condition^{3,4}. The treatment with freeze-dried scorpion venom (redissolved in 0.85 per cent NaCl solution) consisted of: (a) one injection of LD_{25} dose; (b) two injections totalling LD_{40} at a 48-h interval; and (c) three injections administered at 48-h intervals totalling LD_{50} dosage. In immune reaction tests, the highest resistance of treated locusts to challenge doses of scorpion venom (2 to 3 × LD_{50}) was found 24 h after the final sub-lethal injection⁵. Haemolymph for specific gravity measurement was therefore extracted 24 h after the final sub-lethal injection.

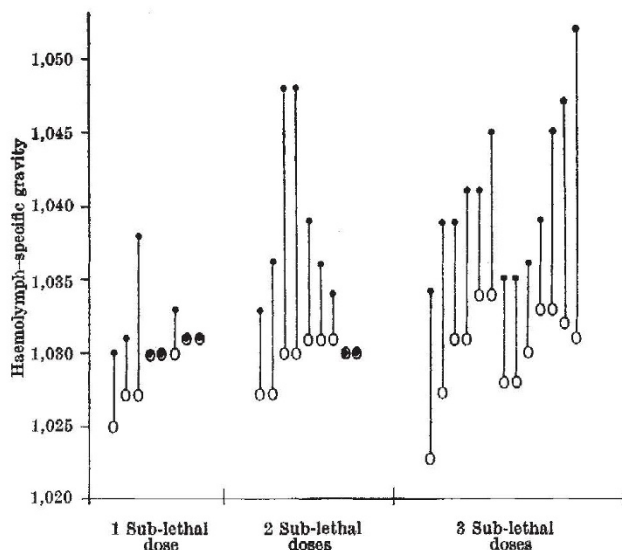


Fig. 1. Specific gravity of haemolymph removed from female locusts treated (●) with scorpion venom, compared with that of untreated female locusts (○)

The specific gravity of haemolymph extracted from the various batches of locusts was determined by the gradient column method of Linderström-Lang as modified by Patton¹. In these tests, 500-ml. graduated cylinders of the kerosene-bromobenzene mixtures were employed.

The results shown in Fig. 1 clearly demonstrate that the specific gravity of haemolymph from treated female locusts is higher than that of untreated female locusts; the difference can be noted even after single injections, although on multiple injections of venom the effect is most pronounced. Specific gravity measurements of the haemolymph extracted from treated male locusts, in contrast, failed to show any increase over that of the haemolymph of the control males.

Since the results given in Fig. 1 relate only to haemolymph extracted from the locusts 24 h after the final sub-lethal venom treatment, the duration of the effect on the treated haemolymph was determined. Patton¹ notes, as already mentioned, that the rise in specific gravity of the haemolymph of sodium arsenate-treated *G. mellonella* larvae is transitory and that the values gradually return to normal in those individuals surviving 3 h after the injection of the LD_{50} dose.

Table 1. SPECIFIC GRAVITY OF HAEMOLYMPH FROM FEMALE LOCUSTS EXTRACTED 3-14 DAYS AFTER TREATMENT WITH THREE SUB-LETHAL DOSES OF SCORPION VENOM

Haemolymph removal (days after final sub-lethal dose)*	Specific gravity of Treated locusts	Untreated locusts
3	1.040	1.037
4	1.043	1.037
5	1.045	1.037
6	1.053	1.038
7	1.045	1.038
8	1.045	1.038
9	1.042	1.037
10	1.044	1.029
11	1.043	1.036
12	1.043	1.036
14	1.043	1.036

* Treatments consisted of 3 sub-lethal doses of 0.8-1 µg scorpion venom per g weight locust (LD_{50} had been previously determined to be 2.42 µg/g female⁵).

† Average values of 3 extracts (each containing pooled haemolymph from 4 locusts) from every treatment batch.

Tests of the duration of the effect of 3 sub-lethal injections of scorpion venom on the specific gravity of female locust haemolymph are summarized in Table 1. Haemolymph was extracted 3-14 days after the final sub-lethal dose. It can be seen that the specific gravity of treated haemolymph remains significantly higher than control values up to a fortnight after venom treatment.

The cause of the change in the specific gravity of the haemolymph of treated locusts remains to be investigated. This phenomenon does not seem to be related to the acquired immune response shown by treated locusts⁵, since the haemolymph of male locusts shows no increment in specific gravity, while the males express the immune response at the same level as do the females.

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¹ Patton, R. L., *J. Insect Physiol.*, **8**, 537 (1962).

² Siakotos, A. N., *J. Gen. Physiol.*, **43** 999 (1960).

³ Kamon, E., and Shulov, A., *J. Insect. Pathol.*, **5**, 206 (1963).

⁴ Hunter-Jones, P., *Rearing and Breeding Locusts in the Laboratory*, 12 (Anti-Locust Research Centre, London, 1961).

⁵ Kamon, E., and Shulov, A., *J. Insect. Pathol.* (in the press).