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RESEARCH NOTE

CARBOHYDRATE ANALYSIS IN SPIDER HEMOLYMPH OF SELECTED LYCOSID AND ARANEID SPIDERS (ARANEAE: LYCOSIDAE AND ARANEIDAE)

The literature gives incomplete and conflicting information concerning the carbohydrate composition of hemolymph in the Araneae. Various analyses of hemolymph components have been reported on several spiders including: the spider *Cupiennius salei* (Keyserling 1877) by Loewe et al. (1970); the theraphosid *Aphonopelma hentzi* (Girard 1854) by Stewart & Martin (1970); the large orb weaver *Nephila madagascarensis* (Vinson 1863) by Rakatovao & Ratsimamanga (1975); the spider *Hexathele hochstetteri* Ausserer 1871 by Bedford (1977); the araneids *Araneus gemma* (McCook 1888) and *Argiope trifasciata* (Forskål 1775) by Cohen (1980); selected species of the family Lycosidae by Punzo (1982); sparassids, pisaurids, and amaurobids by Punzo (1983); the theraphosid *Eurypelma californicum* Ausserer 1871 by Schartau & Leidescher (1983); hemolymph inorganic ions in 15 spiders and six scorpion species by Burton (1984). Punzo (1989) studied four species of mygalomorphs *Bothriocyrtum californicum* (O.P. Cambridge 1874), *Aphonopelma echinum* (Chamberlin 1940), *Euagrus comstocki* Gertsch 1935, and *Atypus bicolor* Simon 1836.

With regard to the identity of carbohydrates present in the hemolymph, Rakatovao & Ratsimamanga (1975) and Bedford (1977) identified trehalose and glucose, while Schartau & Leidescher (1983) identified only glucose. Loewe et al. (1970) and Stewart & Martin (1970) hypothesized the presence of trehalose because their data indicated sugars other than glucose present. The studies by Cohen (1980) and Punzo (1982, 1983, 1989) quantified carbohydrate concentration differences among species but did not identify the carbohydrate(s) present. The studies by Loewe et al. (1970) and Schartau & Leidescher (1983) reported the presence of circulating glycoproteins, while the other studies did not test for these carbohydrates. The findings of Loewe et al. (1970), Stewart & Martin (1970), and Bedford (1977) indicate individual variation within conspecifics, while the other studies report total carbohydrate concentration within a given species.

In an attempt to clarify the carbohydrate composition of spider hemolymph, this study was conducted using *Argiope aurantia* Lucus 1833, *Hogna carolinensis* (Walckenaer 1805), *Arctosa littoralis* (Hentz 1844), and *Rabidosa rabida* (Walckenaer 1837). Spiders used in this study were collected in north-central Texas. Species were selected based on availability, size, and volume of hemolymph recovered from each individual. Voucher specimens of species used in the study are on deposit at the American Museum of Natural History (AMNH), New York. The lycosids were placed in metal cans (10 cm × 14 cm) which contained a small amount of soil, offered crickets and water, and were tested within 48 h of collection. Since *A. aurantia* is an orb weaver, the specimens were removed from the webs, brought to the laboratory, and tested. The spiders were anesthetized with carbon dioxide and placed on a surgical restraint following Randal (1980). The legs were severed at mid-femur and hemolymph was collected with capillary tubes, which were stored in 400μl microfuge tubes. The tubes were labeled and centrifuged at 12,000 × g for 10–12 min. The resultant cell-free hemolymph was refrigerated at approximately 3 °C and analyzed within 72 h.

Assays included total anthrone reaction to carbohydrates in untreated hemolymph and anthrone reaction after protein precipitation (Dubois et al. 1956) and specific enzyme digestion with glucose oxidase (Fleming & Pegler 1963) with and without trehalase digestion.

A variation on the phenol-sulfuric acid test, using an addition of trichloroacetic acid (TCA) to bring the sample to a total of 5% TCA, was used to test for free carbohydrate. This new sample at 5% TCA was centrifuged 8–10 min at 12,000 × g, and 200μl of the resultant clear supernatant was retained for total carbohydrate analysis.

Solutions of known concentration were used to plot standard curves from the resultant absorbance data at 470 nm in order to quantify total carbohydrates. Each group of spider hemolymph was analyzed against standards prepared at the same time. There was a range of variation among individuals for total carbohydrate concentration. As an example, *R. rabida* exhibited a range from 3.54μg to 45.8μg of carbohydrates per 10μl of hemolymph. The variation in the amount of total carbohydrate present was a consistent feature of the data (C.V. = 44).

Total glucose was determined from comparisons of glucose oxidase activity of solutions of known concentration. Considerable individual variation occurred in the amount of glucose present in 5μl hemolymph samples. Absorbance data for each control group varied. With regard to different sets of individuals, the data were qualitatively based on variance of these standard absorbencies.

The amount of glucose present after digestion with trehalase was determined for *A. aurantia.* The total amount of glucose present increased following treatment of the hemolymph with trehalse. Based on a two point ANOVA, glucose present after trehalase digestion did not differ from the total amount of glucose present before treatment ($P > 0.2$). For example, *A. aurantia* hemolymph contained an average of 5.77 ±2.12μg of glucose per 5μl hemolymph without trehalase treatment and an average of 7.89 ±3.11μg glucose per 5μl hemolymph after trehalase treatment.

Total carbohydrate analysis after protein precipitation with TCA was performed on 16 *A. aurantia* and two *R. rabida.* The presence of TCA altered the color yield for the glucose standard solutions. Thus, standards were treated the same way as the hemolymph samples and analyzed simultaneously (TCA and control samples) to correct for this effect. In comparing the total carbohydrates with and without TCA treatment, there was a two-fold decrease in the amount of carbohydrates present in all but one of the 18 specimens tested. Free carbohydrates accounted for less than 50% of the carbohydrates present in the majority of the samples.

In summary, glucose is the only detectable carbohydrate present in the hemolymph. The differences between total carbohydrate and total glucose are small and may be accounted for by the change in color yield of the standards from test to test. Thus, the absorbance data sets are not readily comparable due to the shift in relative color yield. The increase in the amount of glucose present after trehalase treatment is not significant. Data indicate a high degree of individual variation among individuals in the concentration of glucose in the hemolymph. These varying concentrations may reflect the physiological conditions of the animal and the environmental stresses that are placed upon it (Clarke 1979). The data support the observation noted by Loewe et al. (1970) and Schartau & Leidescher (1983) that glucose exists partially in glycoproteins. Free glucose accounts for less than 50% of the total glucose present in this study. More work is needed to describe the existence of a hemolymph glycoprotein in the Araneae. The extensive physiological work done on the class Insecta should serve as a model for investigations in other classes of arthropods.

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