SHORT COMMUNICATION

PROPERTIES OF THE HAEMOLYMPH OF THE CORN EARWORM*†

R. L. BURTON,1 DARREL G. HOPPER,2† JOHN R. SAUER2
and JOHN H. FRICK2

1Entomology Research Division, Agricultural Research Service, U.S.D.A.; and
2Department of Entomology, Oklahoma State University, Stillwater, Oklahoma
74074
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Abstract—1. We determined values for several properties of the haemolymph of the corn earworm, Heliothis zea (Boddie) and compared them with previously reported results for Lepidoptera.
2. The concentrations of Na, K, Mg, Ca, Cl were found to be about 23, 37, 68, 3 and 25 mM.
3. The total osmolality was about 338 mOsmole.
4. The total protein concentration with respect to bovine albumin was about 92 μg/μl, or about 9 per cent (w/w).
5. Haemolymph volume was about 233 μl, which implies a haemolymph volume/larval weight percentage of about 33.
6. Haemolymph pH was 6-5.

INTRODUCTION

We examined several properties of the haemolymph of the corn earworm, Heliothis zea (Boddie). In particular, we determined values for pH, volume, osmolality and concentrations of sodium, potassium, magnesium, calcium, chloride and total protein. This study was made to aid us in the development of a physiological saline solution for use with in vitro studies of the movement of molecules and ions across the midgut epithelium of this insect.

MATERIALS AND METHODS

A colony of corn earworms was maintained in the laboratory by using the rearing technique developed by Burton (1969). However, this procedure was modified to produce only 100 insects/day, and CSM diet (Burton, 1970) was used rather than bean diet.

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† Mention of a proprietary product does not constitute an endorsement of this product by the U.S. Department of Agriculture.
‡ Present address: Department of Biochemistry, Oklahoma State University, Stillwater 74074.
Samples of haemolymph were taken from 11- to 13-day-old last instar larvae that were 3–4 days away from pupation. This age corresponds with the peak in the larval weight-age curve reported by Jones & Lewis (1971); however, the average weight of our insects, 704 mg, was significantly greater than their peak value of 615 mg. The sampling procedure was as follows: an insect pin was used to puncture the cuticle at the base of the second pair of prolegs. Then clear haemolymph was drawn with a glass capillary tube from the pool that formed between the prolegs. Care was taken to avoid contamination from tissue which, upon occasion, appeared at the puncture, and whenever the gut was accidentally pierced, the larva was discarded. Measurements of pH were made immediately after the haemolymph was withdrawn by placing 25–100 µl in the bottom of a 1-ml beaker and inserting a combination microelectrode. Tapered capillary tubes were used to obtain the samples required in the measurement of osmolality. For all other determinations, 25 µl of haemolymph were mixed into a premeasured volume of distilled water (5 ml for most tests), and the capillary tube was rinsed once with the diluted solution.

The sodium, potassium, magnesium and calcium were determined by atomic absorption (Beckman 440 Spectrophotometer®) and the chloride analysis was done by the coulombic silver chloride precipitation method (Fiske–Marius Micro Chloro-o-counter®). The biuretic protein test (Lowry et al., 1951) was employed with bovine albumin as a standard to assess the protein content. The osmolality was established by freezing-point depression with a Clifton Technical Physics biological cryostat-nanoliter osmometer®. The 14C-inulin dilution method was used to determine blood volume (Wharton et al., 1965) as follows: A 10-µl aliquot of 14C-inulin solution (~95,000 counts/min) was deposited into the haemolymph inside a first proleg by inserting a small hypodermic needle into the segment immediately in front of the first set of prolegs. The needle was inserted through fat body to help prevent bleeding on withdrawal and was carefully positioned so that the tip was visible through the integument at the base of a first proleg to insure injection into the haemocoel. Then any pressure exerted by holding the larva was eased before the needle was withdrawn. In this way, zero to an infrequent maximum of about 3 µl of haemolymph was lost. After 45–60 min, a 25-µl sample of the haemolymph was taken and placed in 15 ml of a liquid scintillant described by Wharton et al. (1965). Samples, standards and blanks were then counted with a Beckman LS-100 Liquid Scintillation System®.

RESULTS AND DISCUSSION

The results are presented in Table 1.

The concentrations of sodium, potassium, magnesium and chloride were within the ranges established for other Lepidoptera: 1–40, 15–100, 10–100 and 20–35 mM, respectively (Florkin & Jeuniaux, 1964); the concentration of calcium was slightly low compared with its established range, 5–60 mM (Florkin & Jeuniaux, 1964). The sodium/potassium concentration ratio computed from the best experimental values was 0.63 ± 0.16, which is less than 1 in accordance with previous results for phytophagous Lepidoptera (Chapman, 1969).

Our value for the total protein concentration, 92.1 ± 9.1 µg/µl of haemolymph, was significantly larger than both the average value of 20 µg/µl reported by Florkin & Jeuniaux (1964) for Lepidoptera and the value of 46.25 µg/µl found recently by Shapiro & Ignoffo (1971) for 10-day-old corn earworm larvae. Shapiro & Ignoffo (1971) also found the value of 25.75 µg/µl for 7-day-old larvae; they, as did we, used the Folin phenol reagent (Lowry et al., 1951) with bovine albumin standard. The difference between our value and that of Shapiro & Ignoffo’s may be due to differences in larval age, diet, growth conditions, bovine albumin standards and
### Table 1—Ion and Total Protein Concentrations, Total Osmolality, Volume and pH of the Haemolymph of the Corn Earworm Larva: Average Larval Weight

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Experiment*†</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na], mM</td>
<td>27.6 ± 11.7</td>
<td>22.8 ± 3.6 (5)</td>
<td></td>
</tr>
<tr>
<td>[K], mM</td>
<td>37.8 ± 4.6</td>
<td>36.8 ± 3.4 (10)</td>
<td></td>
</tr>
<tr>
<td>[Mg], mM</td>
<td>60.8 ± 8.0</td>
<td>68.4 ± 2.2 (10)</td>
<td></td>
</tr>
<tr>
<td>[Ca], mM</td>
<td>2.96 ± 0.65</td>
<td>2.74 ± 0.29 (10)</td>
<td></td>
</tr>
<tr>
<td>[Cl], mM</td>
<td>25.8 ± 8.1</td>
<td>25.2 ± 2.2 (10)</td>
<td></td>
</tr>
<tr>
<td>Ion total, mM</td>
<td>155.0 ± 33.1</td>
<td>155.9 ± 11.7</td>
<td></td>
</tr>
<tr>
<td>Total protein†</td>
<td>92.1 ± 9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmolality, mOsmoles/kg H2O</td>
<td>337.8 ± 15.3</td>
<td>704 ± 40 (14)</td>
<td></td>
</tr>
<tr>
<td>Volume, µl</td>
<td>233 ± 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larval weight, mg</td>
<td>704 ± 40</td>
<td></td>
<td></td>
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<tr>
<td>Haemolymph volume percentage (µl/mg) × 100</td>
<td>33.2 ± 3.0 (14)</td>
<td>6.547 ± 0.040 (10)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
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</tbody>
</table>

* The ranges given were determined by the t-test for a 95 per cent confidence interval.
† The parenthesized values are the numbers of larvae tested.
‡ Expressed in terms of the equivalent weight of bovine albumin in µg/µl of haemolymph.

Experimental error. Florkin & Jeuniaux (1964) state that in Lepidoptera the level of haemolymph protein increases during larval life and then decreases at the end of the pupal instar. Our larvae were 11–13 days old.

As for percentage protein (w/w) in the corn earworm haemolymph, we calculated a value of 8.94 ± 0.88 per cent on the basis of our values for the total protein concentration and the haemolymph volume by assuming that the haemolymph density was 1.030 mg/µl (Buck, 1953).

The osmolality corresponded closely to previously reported values for terrestrial insects (Shaw & Stobbart, 1963); just less than half was attributable to the five inorganic ions we determined.

The volume of haemolymph was in the same range as that found for the male American cockroach, Periplaneta americana L. (Wharton et al., 1965) and for an African migratory locust, Locusta migratoria migratorioides (Riche & Sairmarie), during its last nymphal instar (Loughton & Tobe, 1969), and the volume percentage was only slightly lower than that reported for the cockroach by Wharton et al. (1965). We made no correction for tissue absorption, excretion or metabolism of 14C-inulin in our volume determination, though Wharton et al. (1965) found a 7.8 per cent loss in P. americana after 4 hr, and Levenbook (1958) found a loss of less than 5 per cent in the southern armyworm, Prodenia eridania (Cramer), after 12 hr. Since we allowed the injected inulin only 45–60 min to equilibrate before we took our samples (the maximum time for mixing in some insects is 30 min according
to Patton, 1963), our loss of $^{14}$C-inulin was probably less than 5 per cent. Thus, our range of 9 per cent for insect variability—experimental error (computed from the experimental range for the volume of haemolymph shown in Table 1) is larger than the probable correction to be made.

The pH was within the 6–7 range common for insect haemolymph (Chapman, 1969) but contrasts with the alkalinity of the gut contents, the pH of which we found to be 9.3.

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REFERENCES


Key Word Index—Heliothis zea: properties of haemolymph; insect blood; haemolymph composition and characteristics; Lepidoptera: Noctuida; corn earworm.