STUDIES ON THE EXCRETA OF A GRASSHOPPER
(*MELANOPUS BIVITTATUS* SAY.)

BY A. W. A. BROWN

From the Department of Biochemistry, University of Toronto

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The methods hitherto employed in the study of the gross metabolism of insects have been based mainly on the changes in their content of physiologically important substances during development, and on the ability of the organism to grow on selected diets of known composition. There is a third method, extensively employed by mammalian physiologists, namely analysis of excreta; this, as Uvarov (1928) has pointed out, necessarily involves its correlation with the constitution of the food. Very few analyses of insect excreta, however, have been made, and those of any completeness are confined to species of unusually specialized food habits, i.e. the clothes moth (Babcock, 1912), (Holland & Cordebard, 1926), and *Rhodnius prolixus* (Wigglesworth, 1931).

The insect selected for this study was the grasshopper, *Melanoplus bivittatus* Say, an unspecialized phytophagous type, morphologically rather primitive; the diet used throughout the experiment consisted of head lettuce alone, with distilled water *ad lib.* 5000 individuals were raised from egg to adult on this diet, their excreta being frequently collected, freed of fragments of food and exuviae, and placed in a calcium chloride desiccator; about 30 g. were thus obtained. Unless otherwise indicated, standard methods of analysis, such as are used in clinical investigations, were employed (Peters & Van Slyke, 1932).

GENERAL APPEARANCE

The excretory pellets are oblong ellipsoidal in shape, considerably wrinkled, up to 3.5 mm. in length, and red, brown or black in colour; a few pellets occur which are typically large, more friable and coloured a light grey. They are expelled from the rectum under a certain amount of pressure; on expulsion they are moist, but become apparently dry within a few minutes.

Microscopical examination showed the pellets to possess a wrinkled membranous framework, with many folds, projections and bosses, of a grey translucent aspect. Crystalline encrustations, often red and yellow, adhere in patches to the outer surface, being more abundant in the folds. Maceration revealed crystals, cellulose fibres, and spherules (probably starch grains), and longitudinal striae could be seen in the membrane.
The above observations, together with biochemical evidence given below, indicate that these excreta consist of the peritrophic membrane, enclosing the undigested residues, impregnated and encrusted on the outside by the crystalline products of the Malpighian tubules.

**DRY WEIGHT**

The excreta, except where otherwise indicated, were first ground in a mortar for 20 min. Portions were then weighed out for the various analyses, two samples being dried to constant weight in the oven at 110° C. (for 80 hours). The figures were as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial weight</th>
<th>Final dry weight</th>
<th>% dry weight</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5004</td>
<td>1.3885</td>
<td>92.58</td>
<td>92.5</td>
</tr>
<tr>
<td>2</td>
<td>1.5035</td>
<td>1.3900</td>
<td>92.45</td>
<td></td>
</tr>
</tbody>
</table>

Using this factor, the values determined for each constituent were calculated to the basis of mg. per g. dry weight.

**WATER-SOLUBLE FRACTION**

The aqueous extract was a clear brown liquid with a characteristic smell, not unlike that of tea. The brown colour darkens gradually as the pH is made alkaline. If this liquid be considered as mainly urinary in nature, the following figures may be of value:

- pH 6.3 (colorimetric), 6.38 (quinhydrone).
- Titratable acidity: 4 samples titrated to phenolphthalein 5.44, 4.77, 4.44, 4.26 c.c. 0.1N NaOH per g.
- Total solids: 435 mg. per g.

**KJELDAHL NITROGEN**

The figure for nitrogen content of the excreta by this method is approximately 40 mg. per g., lower values being given by the micro-Kjeldahl analysis, for no apparent reason except variation in sampling:

<table>
<thead>
<tr>
<th>mg. N per g.</th>
<th>mg. N per g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macro-Kjeldahl ... 42.4</td>
<td>Micro-Kjeldahl ... 39.2</td>
</tr>
<tr>
<td>43.5</td>
<td>36.7</td>
</tr>
<tr>
<td>43.4</td>
<td></td>
</tr>
</tbody>
</table>

**URIC ACID**

Determinations were made by the colorimetric method of Benedict & Franke. The two main considerations in the analysis of this substance were (a) the slight solubility of the free acid, which is freely soluble as its sodium or lithium salt; (b) the inhibition of its solution by fatty substances removable by ether. The values for uric acid obtained by various methods of treating the excreta are shown in Table I.
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Table I. Uric acid in Melanoplus excreta, using various methods of extraction

Extractions made in volumetric flasks in electric oven, except in C, where Erlenmeyer flasks were used

<table>
<thead>
<tr>
<th>No.</th>
<th>Preliminary treatment</th>
<th>Temp. °C.</th>
<th>Duration of time</th>
<th>Proportion of solvent to amount excreta extracted</th>
<th>Uric acid mg. per g. excreta</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1</td>
<td>Not ground</td>
<td>65</td>
<td>First soak 7½ hours</td>
<td>500 c.c./g.</td>
<td>H₂O</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>Second soak 10 hours*</td>
<td>—</td>
<td>0.4% Na₂CO₃</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.4% Li₄CO₃</td>
</tr>
<tr>
<td>B 4</td>
<td>Ground</td>
<td>65</td>
<td>Soaked for 3½ hours with shaking every ¾ hour</td>
<td>1000 c.c./g.</td>
<td>H₂O</td>
</tr>
<tr>
<td>5</td>
<td>Not ground</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C 6</td>
<td>Ground</td>
<td>60</td>
<td>3 rapid extractions, with continuous shaking, cooled immediately after filtration</td>
<td>250 c.c./g.</td>
<td>0.4% Li₄CO₃</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>—</td>
<td>Method for extraction from snake excrement (Plimmer, 1933)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D 8</td>
<td>Ground and ether-extracted in a Soxhlet apparatus</td>
<td>65</td>
<td>First soak 3 hours with shaking every ¾ hour</td>
<td>400 c.c./g.</td>
<td>H₂O</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>—</td>
<td>Second soak 3 hours</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Amount of uric acid in second soak: in 1, 3.4% of total; in 2 and 3, trace only.

The low value of sample 9 was at first thought to be due to the fact that it had been first oven-dried for over 100 hours, during which period there might have been destruction of uric acid. However, it was found experimentally that samples of crystalline uric acid, exposed to 110°C for periods up to 150 hours, suffered no destruction, as measured by the colour with the reagents of Benedict & Franke.

The above data indicate that the best method for the extraction of uric acid from Melanoplus excreta is to grind the material, extract with ether, and then extract twice with distilled water at 65°C, shaking for 3 hours at each extraction.

UREA

The presence of urea in small quantities in the blood of insects has frequently been reported. Claims for its presence in excreta, although reported by Babcock, and by Holland & Cordebard, for the clothes moth, have been regarded as ill-substantiated (Wigglesworth, 1931); and the uricotelic rule for an animal with a cleidoic egg and terrestrial habit (Needham, 1931) makes its occurrence appear
unlikely. However, its presence was detected in the excreta of *Melanoplus*, and the amount was determined by two different methods:

- Colorimetric urease method of Wakeman & Morrell \( \ldots \) 3·39 mg. urea per g.
- Gravimetric xanthydrol method of Fosse \( \ldots \) 3·34 mg. urea per g.

**AMMONIA**

This catabolite is present only in very small amounts in the aqueous extract of *Melanoplus* excreta. Values for three samples, determined by Permutit adsorption and Nesslerization (method of Folin & Bell), were as follows: 0·70, 0·68, 0·84 mg. per g.

**AMINO NITROGEN**

Evidence for the excretion of amino acids by insects rests upon the identification of leucine by some earlier workers by its crystal form (Ehrenberg, 1914). The amount of amino nitrogen in the aqueous extract of *Melanoplus* excreta was found to be quite considerable. It would appear that insects, like echinoderms and Crustacea, possess an excretory mechanism that is deficient with respect to amino acid retention.

Values given by the colorimetric method of Folin were:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Value (mg. N per g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3·45</td>
</tr>
<tr>
<td>2</td>
<td>1·82</td>
</tr>
</tbody>
</table>

Because of the discrepancy between the two figures, and the fact that alkaloids are stated to give a colour with this reagent (Gortner, 1929), recourse was taken to analysis by the gasometric method of Van Slyke, the reaction being allowed to proceed for 5 min.:

\[
\begin{align*}
\text{Sample 1} & \quad 3'41' \\
\text{Sample 2} & \quad 3'51' \\
\text{Correction for NH}_3 & \quad 0'18 \\
\text{Correction for urea} & \quad 0'10 \\
\text{Final average value} & \quad 3'18 \\
\end{align*}
\]

Qualitative tests for individual amino acids were applied, with negative results, with the exception of doubtfully positive indications of the presence of cystine.

**ARGININE**

The presence of creatine and creatinine in insect excreta has been reported (Babcock, 1912; Wigglesworth, 1931), the latter author considering creatine to be an end-product of metabolism. No evidence for the existence of either of these two compounds in *Melanoplus* excreta was given by Jaffé’s test, which was negative whether applied directly or after boiling with 8 per cent HCl for \( \frac{1}{2} \) or 2 hours.

In view of the growing mass of evidence showing that phospho-creatine is absent as the “phosphagen” of invertebrates, its place being taken by phospho-arginine, the aqueous extract of *Melanoplus* excreta was tested for arginine; this
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substance was found to be present. The values given for two samples by the colorimetric method of Weber (1930) were:

Sample 1 ... ... 0.67 mg. per g.
,, 2 ... ... 0.62

PROTEIN

There is no evidence for the existence of protein in the excreta of Melanoplus. Biuret tests were applied to three samples of aqueous extract, to extracts in 0.5 per cent Na₂CO₃ and 0.1N NaOH, and to the insoluble residues, with entirely negative results.

It had been hoped that undigested protein would fill the gap between the figure for total nitrogen and the sum of those for the individual nitrogenous compounds; the discrepancy therefore still remains.

CARBOHYDRATES

The following observations are preliminary to a fuller investigation.

For the aqueous extract of excreta:

Reducing sugars (Benedict’s 2nd mod.), negative.
Pentose sugars (Bial’s), negative.
Quantitative estimation with Benedict’s copper titration method:
Reducing substances (as glucose), 30.4 mg. per g.

For the solution obtained from acid hydrolysis of water-insoluble fraction of excreta, hydrolysed according to the method for estimation of starch (A.O.A.C. 1925):

Reducing sugars, strongly positive.
Pentose sugars, positive.

ACID RADICALS

Chloride, by the silver precipitation method of Whitehorn 21.0 mg. Cl per g.
Sulphate, gravimetric barium precipitation method ... 30.0 SO₄
Phosphate, colorimetric method of Briggs ... ... ... 19.5 PO₄
Carbonate, micro-modification of method of A.O.A.C. ... 1.2 CO₃
Oxalate, crystals of calcium oxalate noted on evaporation of aqueous extract.

In comparison with the classical analyses of Peligot (1852) for the silkworm, the excretion of carbonate is very low and that of sulphate is unusually high (S : N ratio of 1 : 4).

PHENOLS

The following determinations were made by the molybdotungstate method of Folin and Denis:

In aqueous extract of ether-insoluble fraction:

Free ... ... 9.0 mg. per g.
Total ... ... 10.2

In aqueous extract of ether-soluble fraction,

Free ... ... 4.0 mg. per g.
ETHER EXTRACT

The extract, obtained by treating the ground excreta in a Soxhlet apparatus for 8 hours with freshly distilled anhydrous ether, was olive-green in colour and of the consistency of plasticine. The weights of the extract varied considerably, being much diminished when the excreta had been dried at high temperature for some time; they were as follows:

Weight of ether extract: 35.1, 44.7 and 24.8 mg. per g.

Samples of the extract, analysed for nitrogen by a micro-Kjeldahl process, gave very low figures, averaging 0.31 mg. per g.

PHYLLOBOMBYCIN

A preliminary attempt was made to demonstrate the presence of unchanged chlorophyll in the excreta of the orchard tent caterpillar, *Malacosoma americana* Fab., large quantities of which were on hand. The dark green ether extract was fused and then tested for the presence of ether-soluble magnesium; this was found to be entirely absent.

Fischer & Hendschel (1931) describe the isolation from the excreta of *Bombyx* of an ether-soluble magnesium-free porphyrin derivative which they designated as "phylllobombycin". The ethereal extract of *Melanoplus* excreta, when examined spectroscopically, showed absorption bands very closely approximating the absorption spectrum established for phylllobombycin, as follows:

<table>
<thead>
<tr>
<th>Band</th>
<th>Density</th>
<th>Range</th>
<th>Median</th>
<th>Median for phylllobombycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>Very dense</td>
<td>711–602</td>
<td>701</td>
<td>700.5</td>
</tr>
<tr>
<td>2</td>
<td>Very weak</td>
<td>668–686</td>
<td>674</td>
<td>665.5</td>
</tr>
<tr>
<td>3</td>
<td>Weak</td>
<td>619–606</td>
<td>619</td>
<td>609.9</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>534–530</td>
<td>532</td>
<td>540.0</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>507–498</td>
<td>502</td>
<td>503.2</td>
</tr>
</tbody>
</table>

* Band 1 did not separate into two on dilution.

HIPPURIC ACID

The presence of this substance in insect excreta has been reported by Davy (1854) and Plateau (1873). The following treatment failed to detect any hippuric acid at all. The ether extract of 4 g. of excreta was refluxed with boiling water for 1 hour; the aqueous extract was evaporated to dryness in vacuo, giving a white residue which was almost entirely insoluble in ethyl acetate. Both the fractions—soluble and insoluble in ethyl acetate—when in aqueous solution gave a trace of a creamy precipitate with FeCl₃ at pH 5; this precipitate was apparently soluble in concentrated HCl, but no crystals of hippuric acid were deposited on evaporation of the solvent.
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THE PERITROPHIC MEMBRANE

During microscopical examination and chemical treatment of excreta, especially from individuals fed on synthetic diets, it became increasingly apparent that there was present a water-insoluble element which held the excretory pellet in its characteristic form. It was suspected that this element was the excreted peritrophic membrane. Accordingly, a sample of excreta was hydrolysed in concentrated KOH at 150° C. after the qualitative method of Campbell (1929) for the detection of chitin. After hydrolysis there remained certain hyaline bodies—the skeletons of the excretory pellets—which under the microscope appeared as lengths of tube pinched at either end. They reacted to Campbell's microchemical tests for chitosan as follows:

1. (a) They dissolved only very slightly in 3 per cent acetic acid, with bubbling.
   (b) On addition of 1 per cent H₂SO₄ to the above solution, white needles crystallized out on the edge of the liquid.

2. (a) They gave a strong brown coloration on addition of 0·2 per cent I₂ in KI.
   (b) On removal of excess I₂ and addition of 1 per cent H₂SO₄, the colour turned to red-brown, accompanied by bubbling.
   (c) They dissolved in 75 per cent H₂SO₄ with bubbling.

The intimate relationship of this membrane with the form of the pellet is brought out even better in the case of the excreta of *Malacosoma americana*, which, after treatment with KOH, leave transparent discs with the beautiful six-rayed form characteristic of the faecal pellets.

The quantitative method of Campbell for the estimation of chitin was applied to the excreta of *Melanoplus*, two samples of 1 g. each being treated with 17 per cent KOH at 65° C. for 14 days. The residue was then filtered off in a Buchner funnel on hardened filter paper, washed thoroughly, dried to constant weight and weighed. The weights of this residue were as follows:

| Weight of crude membrane: Sample | 1... | 222 mg. per g. |
|                                | 2... | 214             |

1·5 g. of this alkali-resistant constituent were obtained from excreta residues, and the crude preparation was investigated for its nitrogen content. For estimation by the Kjeldahl method, samples were digested for periods up to 2 days, the intention being to allow for the slow digestion of chitin. The figures proved to be as follows:

<table>
<thead>
<tr>
<th>Period of digestion hours</th>
<th>% nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kjeldahl N</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>48</td>
</tr>
<tr>
<td>Dumas N</td>
<td></td>
</tr>
</tbody>
</table>

1 The residue is contaminated with a small amount of cellulose fibre.
From these figures it appears that the nitrogen is derived from contaminants, and that the membrane is probably nitrogen-free. Further work is projected with a view to establishing the chemical nature of this element, which has up to the present been considered as chitinous (Wigglesworth, 1929).

SUMMARY

1. 30 g. of the excreta of *Melanoplus bivittatus* on a restricted diet of lettuce were obtained from all stages of the insect.

2. Over 20 per cent of the excreta consists of the peritrophic membrane; the negligible nitrogen content of this element indicates that it cannot be chitinous.

3. Enclosed within the membrane are the food residues, and encrusted on the outer surface are the urinary constituents.

4. Uric acid is the predominant nitrogenous catabolite; methods of its extraction from excreta are compared.

5. The presence of urea is demonstrated by two methods, as also is a significant amount of amino nitrogen.

6. Creatine is absent, but arginine is present.

7. The presence of phyllobombycin in the ether extract was indicated by spectroscopic properties.

The author wishes to express his deep gratitude to Dr R. D. Bird and Mr H. W. Moore, of the Dominion Entomological Laboratory at Brandon, Manitoba, for rendering this study possible by supplying the eggs. Hearty thanks are offered to Dr Lionel Farber for micro-Kjeldahl determinations, and to Prof. Hardolph Wasteneys for unfailing help and advice.

REFERENCES


