TRYPTOPHAN METABOLITE EXCRETION BY THE
AMERICAN COCKROACH

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Abstract—1. Three fluorescing materials have been discovered in excreta
from the American cockroach, Periplaneta americana (L.), and found to increase
in concentration as dietary nitrogen increases.

2. These compounds have been identified as kynurenic, xanthurenic and
8-hydroxyquinlalidic acids.

3. Feeding cockroaches antibiotic eliminates 8-hydroxyquinlalidic acid
production, presumably by inhibiting gut microbial dehydroxylation of xanthu-
renic acid.

4. Excreta from wild-type and lavender-eye mutants contains all three
metabolites, while that from pearl-eye mutants contains none of them.

5. Xanthurenic and 8-hydroxyquinlalidic acids may contribute to cockroach
gut tumor formation.

INTRODUCTION

The American cockroach, Periplaneta americana (L.), is a terrestrial insect, and
has been characterized as being a uric acid excreter (Nation & Patton, 1961;
Srivastava & Gupta, 1961; McEnroe, 1966; Bursell, 1967; Corrigan, 1970). An
extensive re-examination of this question in our laboratory has shown that this is
not the case. Under carefully controlled conditions these insects do not excrete
significant quantities of uric acid externally, nor do they excrete a wide variety of
other known insect excretory products (Mullins, 1971; Mullins & Cochran, 1972).
However, we discovered that certain tryptophan metabolites are excreted by this
species. It is the purpose of this paper to report the details of the tryptophan
metabolite findings.

Tryptophan and its derivatives are known to be metabolized extensively by
insects (Gilmour, 1961; Corrigan, 1970). For example, the pathway involving
kynurenine and 3-hydroxykynurenine leads to the synthesis of ommochrome eye
pigments (Butenandt, 1959), although many of the later steps in this pathway are
unknown (Ziegler, 1961). In addition, kynurenine can be converted to a number of
intermediates some of which are excreted. This situation prevails in the silkworm
and the fruit fly where xanthurenic acid is voided (Inagami, 1955; Umebachi &

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549
Tsucthitani, 1955). Because a number of these reactions are pertinent to this paper, they are shown in Fig. 1.

The second major pathway for the metabolism of tryptophan, known primarily from mammals, involves its conversion to 5-hydroxytryptophan, which can subsequently react to form 5-hydroxytryptamine or one of a number of indole derivatives (Corrigan, 1970). This pathway has not been studied extensively in insects and does not appear to be of major importance to the present report, except as a possible alternative pathway in one case.

MATERIALS AND METHODS

Young adult male and female American cockroaches were the principal experimental animals. Cultures of these insects are routinely maintained in our laboratory on a balanced dog food diet containing 24% crude protein. Adults from colonies of the pearl-eye and lavender-eye mutant stocks (Ward & Hammond, 1957; Ross et al., 1964) as well as adults from colonies of the cockroach Leucophaea maderae were also used.

The insects were placed on experimental diets for 1–2 weeks prior to excreta collection. The diets were modified from Haydak (1953) by altering the level of protein or by making certain additions such as 1% tryptophan or 1 or 10% neomycin sulfate. In some experiments casein hydrolysate was substituted for casein protein, and usually the dog food diet was the control.

Excreta were collected from small glass containers housing a specified number of insects. In this way cannibalism could be closely monitored, and its effects eliminated as a possible source of excreta contamination. The excreta were lyophilized, weighed and extracted with 0.6% LiCO₃ at 80°C for 10 min (80 mg excreta/ml). The extracts were centrifuged at 1000 g for 10 min. The supernatants were spotted on 0.25 mm MN 300 cellulose thin-layer chromatographic (TLC) plates. The plates were developed in one of several solvent systems as described in the results. Spots were detected under u.v. light, and R<sub>f</sub> values were calculated for known standards and for unknowns. Tentative identifications were made in this manner. For confirmation, the unknowns were purified by two-dimensional TLC on 1 mm MN 300 cellulose plates using n-butanol–acetic acid–H₂O (8 : 1 : 3 v/v) in one direction and n-butanol–methanol–H₂O–NH₄OH (5 : 3 : 2 : 1 v/v) in the second direction. The spots were detected by u.v. light, removed from the plates, and dissolved in 0.2 N NaOH. Ultraviolet spectra were obtained for these unknowns as well as for appropriate standards using a Beckman DB spectrophotometer. Quantitation of these materials was also achieved spectrophotometrically by making comparisons of standards and unknowns at appropriate absorption maxima. Quantitation of total excreta nitrogen was by a micro-Kjeldahl technique (Schmidt, 1961). Photographs of the TLC plates were taken under u.v. light on Kodachrome II film using Wratten filters 85c, CC10R and 2E (Eastman Kodak Co.).

RESULTS AND DISCUSSION

During the course of the uric acid studies (Mullins, 1971), it was noted that three u.v. fluorescing materials are present in American cockroach excreta. As the dietary protein level of the experimental animals was increased over a range of 5 to 91 per cent, higher concentrations of the fluorescing materials were evident, suggesting that they might be nitrogenous in nature. Analysis of excreta extracts, using TLC with three different solvent systems and appropriate standards, has allowed the identification of these materials (Table 1). They are xanthurenic, kynurenic and
**Table 1—Comparison of TLC* R<sub>f</sub> values of known standards and fluorescing materials found in the excreta of *P. americana**

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>Isobutyric acid–H&lt;sub&gt;2&lt;/sub&gt;O 4:1 (v/v)</th>
<th>n-Propanol–2% NH&lt;sub&gt;4&lt;/sub&gt;OH</th>
<th>n-Butanol–methanol–NH&lt;sub&gt;4&lt;/sub&gt;OH 60:20:20:1 (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Standard Unknown</td>
<td>Standard Unknown</td>
<td>Standard Unknown</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>0.37 0.37</td>
<td>0.54 0.52</td>
<td>0.32 0.31</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>0.54 0.54</td>
<td>0.77 0.77</td>
<td>0.62 0.64</td>
</tr>
<tr>
<td>8-Hydroxyquinaldic acid</td>
<td>0.76 0.76</td>
<td>0.68 0.67</td>
<td>0.54 0.55</td>
</tr>
</tbody>
</table>

* On 0.25 mm MN 300 cellulose plates.

**Metabolism of Tryptophan**

![Chemical diagram of tryptophan metabolism](image)

**Fig. 1.** Metabolism of tryptophan, adapted from Gilmour (1961), representing a generally accepted scheme of tryptophan metabolism in insects.

8-hydroxyquinaldic acids, all of which are considered to be metabolites of tryptophan (Fig. 1). Confirmation of the identifications was obtained from u.v. spectra of the unknowns after purification by two-dimensional TLC and in comparison with known standards. The u.v. spectra of standards and unknowns agreed well in all cases. Absorption maxima were 243, 247 and 268 nm for xanthurenic, kynurenic and 8-hydroxyquinaldic acids, respectively.

Since these materials have not previously been shown to be cockroach excretory products, it was considered important to determine their quantities. This was also
done spectrophotometrically at the absorption maxima in comparison with known quantities of standards. Specifically, results were obtained from excreta voided by males and females maintained on dog food (24 per cent crude protein) and 42 per cent casein protein diets (Table 2). The data show that especially xanthurenic and 8-hydroxyquinaldic acids increase as the dietary protein increases. In these experiments recovery of standards was: xanthurenic acid, 87 per cent; kynurenic acid, 82 per cent; and 8-hydroxyquinaldic acid, 69 per cent. After appropriate allowances for these losses, it was shown that in aggregate these materials account for from 1 to 3 per cent of the total excreta nitrogen.

Table 2—Quantitation of the fluorescing materials present in the excreta from insects placed on two different diets

<table>
<thead>
<tr>
<th>Sex</th>
<th>Diet</th>
<th>Xanthurenic acid</th>
<th>Kynurenic acid</th>
<th>8-Hydroxyquinaldic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Dog food (24 per cent crude protein)</td>
<td>1.4 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>42 per cent casein protein</td>
<td>3.2 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Females</td>
<td>Dog food (24 per cent crude protein)</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>42 per cent casein protein</td>
<td>3.0 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>3.9 ± 0.2</td>
</tr>
</tbody>
</table>

* See text for procedural details.

Xanthurenic and kynurenic acids are not particularly unusual compounds in insects. However, to the best of our knowledge free 8-hydroxyquinaldic acid has not previously been reported, although its methyl ester was isolated and identified from the prothoracic defensive scent glands of the beetle Ilybius fenestratus (Schildknect et al., 1969, 1971). In mammals, 8-hydroxyquinaldic acid has been found in the urine of rats (Takahashi & Price, 1958) and in the feces of rabbits and rats, where its occurrence is apparently attributable to the anaerobic action of gut microflora in the dehydroxylation of xanthurenic acid (Kaihara & Price, 1963; Booth et al., 1965; Tamada et al., 1967).

To explore this situation in cockroaches experiments were conducted on wild-type and two eye-color mutants. Neomycin sulfate was fed to the insects in different concentrations in order to disrupt normal gut microbial activities (Kaihara & Price, 1963; Booth et al., 1965; Tamada et al., 1967). A 42 per cent casein protein diet and a 42 per cent casein hydrolysate diet were used as controls. Experimental diets (42 per cent casein protein) were spiked with 1 per cent L-tryptophan and 0, 1 or 10 per cent neomycin. No reduction in food consumption or egg production occurred, and mortality was unaltered by the addition of neomycin.

The results (Fig. 2) show that wild-type females maintained on the 42 per cent casein protein diet (CP) excreted significant amounts of 8-hydroxyquinaldic acid.
and much smaller amounts of xanthurenic and kynurenic acids in these particular chromatograms (see Table 2 for quantitation on more extensive data). In contrast, those insects fed on the casein hydrolysate (CH), which contains no tryptophan, produced only barely detectable amounts of any of these materials. This implies that no other dietary amino acid is appreciably metabolized along this pathway.

Wild-type and lavender-eye females fed the 42 per cent protein diet containing 1 per cent tryptophan produced large amounts of all three metabolites and showed a definite decrease in the excretion of 8-hydroxyquinaldic acid as the concentration of neomycin increased from 0 to 10 per cent. The xanthurenic and kynurenic acid spots, however, did not show a correspondingly large change in concentration (Fig. 2). Indeed, at the 1 per cent neomycin level the size of these spots may have increased, while perhaps some decrease occurred at the 10 per cent level. The results obtained from pearl-eye mutants were very different, since the fluorescing materials in their excreta were present either at (Fig. 2, plate 2) or below (Fig. 2, plate 1) the threshold of detection. It is also of interest to note that all females fed diets containing 1 per cent tryptophan did not excrete tryptophan in detectable quantities. This is in spite of the fact that the mutant forms are reported to be blood tryptophan accumulators (Ward & Hammond, 1957; Ross et al., 1964).

These results can be interpreted to mean that (1) Wild-type cockroaches excrete xanthurenic and kynurenic acids under conditions where tryptophan is in excess of their requirements. The excretion of 8-hydroxyquinaldic acid, however, is based on a tryptophan pathway intermediate, probably xanthurenic acid, which is provided by the insect and apparently is dehydroxylated by the gut microflora. (2) Lavender-eye color mutants seemingly do not possess an enzymatic block(s) along the ommochrome synthetic pathway prior to the formation of kynurenic or xanthurenic acids, since both of these materials are excreted in concentrations similar to wild-type individuals. Therefore, it is most likely that the metabolic lesion responsible for lavender-eyes occurs at some point past the synthesis of 3-hydroxy-kynurenine (Fig. 1); (3) On the other hand, pearl-eye mutants may lack one or more enzymes necessary for the conversion of tryptophan or an early intermediate in the pathway leading to the synthesis of the ommochromes.

Of course, other mechanisms, such as altered excretion capabilities in the pearl-eye stock, could be suggested to explain these results. However, since other mutant forms have shown intraorganismal blocks in tryptophan metabolism (Butenandt, 1959) this explanation seems most plausible. It is also of interest to speculate that the pearl eye mutant might shift to the 5-hydroxytryptamine pathway in place of a functioning kynurenine pathway (Corrigan, 1970).

Several of the quinolines associated with this pathway are active mutagens or carcinogens in mice (Bryan et al., 1964; Kuznezova, 1969). Because of their potent biological activity and their prominence in cockroach excreta, similar effects should now be sought from insects. Indeed, it has been found that recurrent nerve severance and anal blockage in the cockroach L. maderae produced tumor-like lesions along the alimentary canal (Taylor, 1969; Taylor & Freckleton, 1969). It was suggested that these lesions may be the result of the accumulation of toxic substances.
amounts of materials produced by gut microbes. In preliminary studies we found that 8-hydroxyquinaldinic acid is excreted along with several other quinolines when L. maderae was fed on diets containing tryptophan. Similarly, in P. americana it was demonstrated that recurrent nerve severance induced gut tumors which were correlated with a hemolymph protein deficiency (Hema, 1966; Hema & Prabhu, 1970). In our work, P. americana, maintained on high protein diets or on diets containing high concentrations of tryptophan for extended periods of time, exhibited high mortality rates. It is suggested that the presence of the quinoline materials in the gut may have contributed to the mortality of these individuals.

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REFERENCES


Fig. 2. The effects of neomycin on the excretion of three tryptophan metabolites by three eye-color forms of the American cockroach. Two MN 300 cellulose thin-layer chromatographic plates were spotted identically, developed in n-butanol-methanol-H₂O-NH₄OH 5 : 3 : 2 : 1 (plate 1) or isobutyric acid-H₂O 4 : 1 (plate 2), and visualized with a u.v. source. CP and CH represent fecal extracts from normal-eye females on 42 per cent casein protein (containing tryptophan) and 42 per cent casein hydrolysate (containing no tryptophan) diets, respectively. The remaining extracts were spotted in groups of three, representing females of the three eye-color types fed on diets containing 42 per cent casein protein, 1 per cent tryptophan and 0, 1 or 10 per cent neomycin.
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Key Word Index—Cockroaches; *Periplaneta americana*; tryptophan; kynurenic acid; xanthurenic acid; 8-hydroxyquinalalic acid; metabolism; excretion.