AN INVESTIGATION INTO THE PHYSIOLOGY OF URATE PELLET EXCRETION BY PARCOBLATTA PULVESCENS (SAUSSURE AND ZEHNTNER) (DICTYOPTERA : BLATTELLIDAE).

by
Hannah Lembke

Thesis submitted to the Graduate Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Entomology

APPROVED:

D. G. Cochran, Chairman and Head

J. L. Eaton

D. E. Mullins

December, 1985
Blacksburg, Virginia
AN INVESTIGATION INTO THE PHYSIOLOGY OF
URATE PELLET EXCRETION BY PARCOBLATTA FULVESCENS
(SAUSSURE AND ZEHNTNER) (DICTYOPTERA : BLATTELLIDAE).

by
Hannah Lembke

(ABSTRACT)

Physiological parameters involved in formed urate pellet excretion by the wood cockroach, Parcoblatta fulvescens were investigated.

Uric acid excretion by last instar juvenile P. fulvescens was studied first. Food consumption, urate and non-urate pellet excretion patterns show a skewed distribution with peak feeding occurring on day six and peak voiding of both pellet types on day seven of a 17.0 ± 2.0 (SD) day ecdysial cycle. The amount of urates excreted is determined by the level of dietary protein (p<0.0001) and is linearly related to protein consumption.

Selective feeding on protein, carbohydrate and cellulose diets by reproductive female P. fulvescens was investigated. Separate consumption patterns exist for each diet. These females did not excrete uric acid.

Urate pellet consumption by reproductive female P. fulvescens was examined in relation to dietary protein and carbohydrate. Urate pellet consumption increases with decreasing protein and increasing carbohydrate levels.
Females that consume urate pellets do not excrete uric acid. These results suggest that urate-containing pellets serve to transfer nitrogen reserves among individuals.

Urate spherules were enzymatically and histochemically identified in the middle and proximal regions of the Malpighian tubules of *P. fulvescens*, *Shawella couloniana* and *Sympleoce hospes*. These spherules are discharged into the hindgut in sufficient quantities to obscure the presence of food residues.

The significance of formed urate pellet excretion is discussed in relation to the nitrogen economy of *Parcoblatta fulvescens*. 
ACKNOWLEDGEMENTS

I wish to extend my appreciation to my major professor Dr. Donald G. Cochran for his advice, criticism and support, both financial and moral. I also thank the members of my committee; Drs. Donald E. Mullins, for technical advice and John L. Eaton, for use of his laboratory space. I am especially grateful to all the members of my committee for their helpful discussions and moral support.

My research would not have been possible without the aid of our technical staff; Mrs. Nancy F. Boles, Mrs. Eva Duncan and Mr. Keith Tignor. Mrs. Duncan has maintained our colonies of Parcoblatta fulvescens. Mrs Boles and Mr. Tignor have rendered invaluable assistance in photography. Mrs. Boles and Mr. David Judge, a fellow graduate student, also deserve special thanks for assisting me with my research. In addition, I wish to express my sincere appreciation to Dr. Mary Ross for letting me use her wonderful Zeiss photomicroscope.

I would also like to extend my thanks to the Jeffress Memorial Trust for their financial support of my work.

I am dedicating my thesis to my daughter

Her hugs and energy have been, and will continue to be, my main motivation for continuing my studies. She is forgiven for the many evenings she has climbed onto my lap and insisted on snuggling instead of letting me work.

iii
# TABLE OF CONTENTS

ABSTRACT ..................................... ii
ACNOWLEDGEMENTS ............................. iii
LIST OF FIGURES ............................... vi
LIST OF TABLES ............................... viii

Chapter

1. INTRODUCTION ............................. 1

2. LITERATURE REVIEW ........................ 5
   Biology of Wood Cockroaches ............... 5
   Uric Acid Excretion by Cockroaches ........ 8
   Fat Body Storage and Mobilization of Urates .. 13
   Paternal Investment of Urate and Transfer of
      Nitrogen in Insects ..................... 16
   Structure and Function of the Insect Excretory
      System ................................ 18

3. GENERAL MATERIALS AND METHODS ........... 25
   Rearing of Parcoblatta fulvescens ........... 25
   Uricase Analysis ......................... 28

4. URIC ACID EXCRETION BY LAST INSTAR JUVENILE PARCOBLATTA
   FULVESCENS (DICTYOPTERA: BLATTELLIDAE) .... 32
   Abstract ................................ 32
   Introduction .............................. 33
   Materials and Methods ........................ 34
   Results ................................ 37
   Discussion ................................ 51

5. DIET SELECTION DURING THE OOTHECAL CYCLE OF
   PARCOBLATTA FULVESCENS ..................... 53
   Abstract ................................ 53
   Introduction .............................. 54
   Materials and Methods ........................ 55
   Results ................................ 57
   Discussion ................................ 62
LIST OF FIGURES

2.1 Diagrammatic summary of the excretory process in terrestrial insects .............. 20

3.1 Individual rearing containers used for Parcoblatta fulvescens .................. 27

4.1 a. Food consumption, urate and normal pellet excretion during the last juvenile instar. Composite graph ....................... 39

4.1 b. Food consumption, urate and normal pellet excretion during the last juvenile instar. Female on 25% protein diet ............. 40

4.1 c. Food consumption, urate and normal pellet excretion during the last juvenile instar. Female on 42% protein diet ............. 41

4.2 a. Food consumption during the last juvenile instar ....................... 42

4.2 b. Total fecal pellet excretion during the last juvenile instar ............... 43

4.2 c. Normal fecal pellet excretion during the last juvenile instar ............... 44

4.2 d. Urate pellet excretion during the last juvenile instar ....................... 45

4.3 Relationship between protein consumption and uric acid excretion during the last juvenile instar .......... 50

5.1 Food consumption by adult female Parcoblatta fulvescens over the reproductive cycle when given a dietary choice ................. 58

6.1 Relationship between dietary protein and urate pellet consumption ............... 73

6.2 Photographs of nitrogen stressed female Parcoblatta fulvescens with and without egg-case consuming formed urate pellets ................. 75

7.1 Diagram of the hindgut of Parcoblatta fulvescens .............................. 87
7.2 Proximal and middle regions of the Malpighian tubules ........................................... 89
7.3 Distal region of the Malpighian tubule containing urate spherules .............................. 91
7.4 Methenamine staining of sectioned Malpighian tubules ............................................... 92
7.5 Malpighian tubule section showing birefringent crystals within the tubule cells ............. 94
7.6 Birefringent crystals contained in large vesicles within the cells of the Malpighian tubules of Parcoblatta fulvescens compared to urate spherules within the lumen .................................................. 96
7.7 Birefringent crystals contained within the lumen of the Malpighian tubule of an older adult male Parcoblatta fulvescens ................................................................. 98
7.8 a,b Rectal contents from an individual not excreting urates .................................... 99
7.8 c,d Rectal contents from a urate excreting individual ................................................... 100
7.9 a,b Freshly collected urate and non-urate containing pellets before and immediately after addition of 0.6% Li₂CO₃ ................................................................. 102
7.9 a,b Urate and non-urate pellets one and two hours after addition of 0.6% Li₂CO₃ ........... 103
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Observed native food sources for <em>Parcoblatta</em> sp.</td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Cockroach species known to void uric acid</td>
<td>11</td>
</tr>
<tr>
<td>4.1</td>
<td>Number of last instar juveniles of either sex within each dietary group used in this study</td>
<td>38</td>
</tr>
<tr>
<td>4.2</td>
<td>Days of onset and cessation of feeding and fecal pellet production</td>
<td>47</td>
</tr>
<tr>
<td>4.2</td>
<td>Effects of sex on food consumption and voiding</td>
<td>49</td>
</tr>
<tr>
<td>5.1</td>
<td>Average daily consumption and total consumption of cellulose, dextrose and protein diets by female <em>Parcoblatta fulvescens</em></td>
<td>60</td>
</tr>
<tr>
<td>5.2</td>
<td>Length of the oothecal cycle for female <em>Parcoblatta fulvescens</em> given a dietary choice compared with females not given a dietary choice</td>
<td>61</td>
</tr>
<tr>
<td>5.3</td>
<td>The amount of uric acid stored within five <em>Parcoblatta fulvescens</em> females given a dietary choice</td>
<td>63</td>
</tr>
<tr>
<td>6.1</td>
<td>Composition of diets used to study urate pellet consumption</td>
<td>70</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

One of the major functions of the insect excretory system is the elimination of excess nitrogen (Maddrell, 1981, Wigglesworth, 1972). The most widely encountered nitrogenous excretory product in insects is uric acid¹ (Bursell, 1967; Cochran, 1975). In general, terrestrial insects are uricotelic and aquatic insects are ammonotelic (Needham, 1938). However, major exceptions to this pattern exist. In 1972, Cochran and Mullins reported that uric acid is not a major nitrogenous excretory product in cockroaches. This was contrary to earlier reports and a general assumption that this compound is excreted by cockroaches (Nation and Patton, 1961; Srivastava and Gupta, 1961).

Cochran (1973) examined the excreta from twenty cockroach species for the presence of uric acid. Only three were found to void it: Symploce hospes, Shawella couloniana, and Parcoblatta fulvescens. These cockroaches void urates in white formed pellets that are separate and distinct from the normal brown fecal material. The urate pellets are compact and show indentations from the rectal pads. This

¹ Unless specifically noted, the terms uric acid and urates will be used interchangeably since the exact form of this compound and its association with proteins and cations such as H⁺, K⁺, Na⁺, NH₄⁺, Ca²⁺ and Mg²⁺ are frequently unknown.
type of packaging of urates is different from the normal insect pattern of mixing the food residues from the midgut with the urine discharged from the Malpighian tubules (Wigglesworth, 1972).

Although uric acid has not been detected in the Malpighian tubules of cockroaches (Kilby, 1963; Nation and Patton, 1961; Srivastava and Gupta, 1961, Wigglesworth, 1972), those species studied are not urate excretors. It has been postulated that in Parcoblatta fulvescens and other urate excretors the urates may be secreted by the Malpighian tubules or the hindgut (Cochran, 1973, 1985). Since large amounts of urate are apparently produced in a short time period, it has also been suggested that urate excretion represents a regulatory mechanism (Cochran, 1973, 1981, 1985; Cochran and Mullins, 1982).

The level of dietary nitrogen relative to metabolic demand has been determined to be one of the controlling factors in the production of urate pellets (Cochran, 1981). Egg-laying females and juveniles did not excrete urates when dietary-nitrogen levels were below 3.0% and 1.5%, respectively (Cochran and Mullins, 1982). As dietary nitrogen was increased over these threshold levels, the amount of urate produced also increased.

In light of this unusual mechanism for the handling of urates, this study was undertaken to further examine physiological factors involved in this process and to define
a possible role for the urate pellets in the biology of *Parcoblatta fulvescens*. The initial aims of this study were to determine the mechanism of formation of the urate pellets and to examine the kinetics of urate transport. An initial assumption was made that the Malpighian tubules are not involved in urate secretion and that this process occurs in the hindgut, thus making this type of study feasible. However, two major findings necessitated a reevaluation of the objectives. These are: 1. that uric acid occurs in the Malpighian tubules of *Parcoblatta fulvescens* and 2. that adult females of this species on a high dextrose, low nitrogen diet will consume unmodified urate pellets.

In view of the facts just mentioned I have approached this study with the following objectives in mind:

1. To examine the daily pattern of uric acid excretion in the preimaginal instar.

2. To evaluate the ability of adult females to selectively feed on protein, carbohydrate and cellulose diets during the oothecal cycle.

3. To investigate the consumption of urate pellets by reproducing females and the effects of dietary protein and carbohydrate on urate pellet consumption.

4. To examine the histology of the Malpighian tubules in both transporting and non-transporting individuals and conclusively demonstrate the presence of uric acid within the tubules.
The ultimate goal of this study was to improve our understanding of this unusual mechanism for dealing with urates and on the biology of urate-voiding species.
Biology of Wood Cockroaches

The fulvous wood cockroach, *Parcoblatta fulvescens*, is a typical wood-dwelling species. It is widely distributed in the eastern, southern, and central United States (Roth and Willis, 1960; Wendelken and Barth, 1971). Wood cockroaches of the genus *Parcoblatta* are usually found in leaf litter (Wendelken and Barth, 1971), and may be found in habitats ranging from densely wooded areas to short grassy areas near small streams (Lawson, 1967). *Parcoblatta pennsylvanica* has also been reported to live in hollow trees, under loose bark, in wood piles and in crevasses in rural buildings (Rau, 1940). Additional collection notes on *Parcoblatta* species may be found in Hebard (1917), Roth and Willis (1960), and Lawson (1967). In a community of several *Parcoblatta* species, there is a spacial distribution of the species and of the sexes within a given species (Gorton, 1980). The wood cockroaches are considered accidental pests, often being brought into dwellings with groceries or wood (Cornwell, 1968).

The biology of *Parcoblatta* has been most intensely studied in *P. pennsylvanica* (Rau, 1940). The total life span of this species is approximately one year. The
oothecal cycle ranges from five to nine days with the egg cases being carried from one to three days. Laboratory studies on *P. pennsylvanica* indicate that the egg-case cycle averages eight days (Cochran, ms. in preparation). Although Rau (1940) reported that the egg cases are dropped unconcealed, the egg cases of *P. pennsylvanica* and *P. virginica* are typically oviposited in a hole, actively dug by the female, in moist earth (McKittrick, 1964). The egg cases hatch in 30 to 41 days with the majority hatching in 32 to 36 days. Almost 100% of the young emerge (Rau, 1940). The egg case is subject to desiccation, but may be able to absorb sufficient water in a humid environment (Cornwell, 1968). Cornwell (1968) also suggested that since the egg case is dropped long before hatching, it may be able to overwinter. Infertile egg cases are produced in the absence of males in *P. pennsylvanica* (Rau, 1940; Cochran, personal communication) and *P. fulvescens* (Lembke, personal observation).

Young nymphs appear in the spring and become increasingly abundant throughout the summer (Gorton, 1980). Rau (1940) suggested that there are five to seven molts during the life of *P. pennsylvanica*, and the duration of the intermolt becomes progressively longer as winter approaches. Overwintering nymphs mature from early spring through summer (Rau, 1940; Cornwell, 1968; Gorton, 1980).
Adult wood cockroaches show a strong sexual dimorphism (Rau, 1940; Wendelken and Barth, 1971). Adult males are fully winged and may be capable of flying up to 100m (Cornwell, 1968). In Parcoblatta fulvescens, adult males are typically straw colored with a pale margination on the lateral edges of the prothorax. Females have short, non-functional, lobed wings that rarely extend past the second abdominal tergite. The wings are frequently light brown and the prothorax is typically darker, but not nearly as dark as the abdominal segments. Both the wings and prothorax of the adult females have pale lateral margins that are much more pronounced than in males. Juveniles are invariably dark brown without the lateral margination of the thorax. Although young nymphs do not readily exhibit the sexual dimorphism of adults, the lateral edges of the meso- and meta-thoracic tergites of preimaginal instar males typically extend over the second or third abdominal segments, whereas preimaginal females exhibit the pattern of younger nymphs.

Adult male wood cockroaches appear at about the same time in late spring as adult females, but become nearly extinct long before the females (Gorton, 1980). Some activity may be observed during the day, but these species are most active at night (Wendelken and Barth, 1971). Mating normally occurs between two and seven a.m. (Cochran, personal communication). Courtship behavior in males is apparently released by a female sex pheromone which
initiates oriented movement towards the female (Wendelken and Barth, 1971). The male displays by wing raising in the vicinity of a female. It has been suggested that this activity disperses a male sex pheromone which releases the behaviors of mounting and feeding on tergal secretions by the female. Mating occurs on the tenth day following maturation on average and the first egg case is deposited five days after mating (Cochran, personal communication).

The food sources for Parcoblatta fulvescens have not been fully determined, but a list of materials which individuals of several Parcoblatta species have been observed to feed on is given in Table 2.1. The data presented suggest that wood cockroaches are capable of feeding on a variety of material that may contain either very high or very low levels of nitrogen. Although much information is lacking on the biology and ecology of the wood cockroaches, one of the most interesting features of their biology is the excretion of formed-urate pellets that are separate and distinct from the normal-fecal pellets.

Uric Acid Excretion by Cockroaches

It had been assumed for many years that cockroaches excrete uric acid as their major nitrogenous excretory product (Miall and Denny, 1886; Nation and Patton, 1961; Srivastava and Gupta, 1961, Wigglesworth, 1972). However,
<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Food</th>
<th>Authors, year</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. lata</em></td>
<td>males</td>
<td>sap from a wounded tree</td>
<td>Gorton, 1980</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>flower petals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cambium layer from a wood chip</td>
<td></td>
</tr>
<tr>
<td><em>P. pennsylvanica</em></td>
<td>males and</td>
<td>mushrooms</td>
<td>Gorton, 1980</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>sap from a wounded tree</td>
<td></td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>mammalian, probably coyote, fecal matter</td>
<td></td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>spittle from a cercropid nymph</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>attacked a weakened <em>Camponotus pennsylvanicus</em> worker ant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Polistes</em> larva</td>
<td>Rau, 1940</td>
</tr>
<tr>
<td><em>P. uhleriana</em></td>
<td>undetermined</td>
<td>aphid honeydew</td>
<td>Guthrie and Tindall, 1968</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>moss on a tree trunk</td>
<td>Gorton, 1980</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>mushrooms</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>bird feces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mammalian cartilage</td>
<td></td>
</tr>
<tr>
<td><em>P. virginica</em></td>
<td>undetermined</td>
<td>aphid honeydew</td>
<td>Guthrie and Tindall, 1968</td>
</tr>
<tr>
<td></td>
<td>males and</td>
<td>mushrooms</td>
<td>Gorton, 1980</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nymphs</td>
<td>decaying plant debris</td>
<td>Cochran and Mullins, 1982</td>
</tr>
<tr>
<td></td>
<td>(from gut contents)</td>
<td>remains of an insect carcass</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>possibly mushroom</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>or fungal material</td>
<td></td>
</tr>
</tbody>
</table>
in the early 1970's, it was determined that uric acid is not excreted by the American cockroach, *Periplaneta americana* (Mullins, 1971; Mullins and Cochran, 1972). Subsequent studies showed that uric acid is not a major excretory product in most other cockroach species studied (Mullins and Cochran, 1972, 1976; Cochran, 1973, 1976, 1979, 1981; Cochran and Mullins, 1982). In addition, it was found that dietary uric acid is the probable source of voided urates in many species of cockroaches (Mullins and Cochran, 1972, 1973; Cochran, 1976). Ammonia was found to be the major nitrogenous excretory product in *Periplaneta americana* (Mullins and Cochran, 1973a). Three tryptophan metabolites, kynurenic, xanthurenic and 8-hydroxyquinoidalic acids, were also present in the excreta (Mullins, 1971; Mullins and Cochran, 1973a, 1973b).

To date, only 17 of 80 cockroach species examined have been shown to void uric acid (Table 2.2). All of these species belong to the family Blattellidae, subfamilies Blattellinae and Plecopterinae. One should note, however, that not all members of these two subfamilies void urate. Two major patterns for urate voiding exist in these species. *Ishnoptera* spp. void urates admixed with the fecal material in the normal insect voiding pattern (Cochran 1979, 1981). The remainder void uric acid in formed white pellets that distinctly show the indentations of the rectal papilla (Cochran, 1973, 1976, 1979, 1981). The voiding patterns of
Table 2.2  Cockroaches of the Family Blattellidae Known to Void Uric Acid

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of Voiding</th>
<th>Author (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blattellinae:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ischnoptera deropeltiformis</em></td>
<td>admixed with fecal pellets</td>
<td>Cochran (1979)</td>
</tr>
<tr>
<td><em>I. rufa debilis</em></td>
<td>&quot;</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>I. rufa rufa</em></td>
<td>&quot;</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>Parcoblatta americana</em></td>
<td>formed pellets</td>
<td>Cochran (1979)</td>
</tr>
<tr>
<td><em>P. fulvescens</em></td>
<td>&quot;</td>
<td>Cochran (1973)</td>
</tr>
<tr>
<td><em>P. lata</em></td>
<td>&quot;</td>
<td>Cochran (1979)</td>
</tr>
<tr>
<td><em>P. pennsylvanica</em></td>
<td>&quot;</td>
<td>Cochran (1976)</td>
</tr>
<tr>
<td><em>P. virginica</em></td>
<td>&quot;</td>
<td>Cochran (1979)</td>
</tr>
<tr>
<td><em>P. uhleriana</em></td>
<td>&quot;</td>
<td>Cochran (1979)</td>
</tr>
<tr>
<td><em>Shawella couloniana</em></td>
<td>&quot;</td>
<td>Cochran (1973)</td>
</tr>
<tr>
<td><em>Symphloce hospes</em></td>
<td>&quot;</td>
<td>Cochran (1973)</td>
</tr>
<tr>
<td><strong>Plecopterinae:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eudromiella</em> sp.</td>
<td>formed pellets</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>Euphyllodromia angustata</em></td>
<td>&quot;</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>Lattiblattella inornata</em></td>
<td>&quot;</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>Nahublatta fraterna</em></td>
<td>&quot;</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>N. nahua</em></td>
<td>&quot;</td>
<td>Cochran (1981)</td>
</tr>
<tr>
<td><em>Riatta</em> sp.</td>
<td>?</td>
<td>Cochran (1981)</td>
</tr>
</tbody>
</table>
the Blattellinae and Plecopterinae appear similar, but differ in their capacity to void urates (Cochran, 1985). Urate content of whole excreta of the two groups are 10-15% and 0.5-3.0%, respectively (Cochran, 1976, 1981).

The voiding of urates by P. fulvescens was first demonstrated by Cochran (1973). The level of dietary nitrogen relative to metabolic demand was later determined to be one of the controlling factors in the production of urate pellets (Cochran, 1981). Egg-laying females and juveniles did not produce urate pellets when dietary nitrogen levels were below 3.0% and 1.5%, respectively (Cochran and Mullins, 1982).

As mentioned previously, uric acid is not a major nitrogenous excretory product in most cockroach species studied (Mullins and Cochran, 1972, 1976; Cochran, 1973, 1976, 1979, 1981; Cochran and Mullins, 1982). However, to date we can recognize four major strategies which seem to have developed in cockroaches for handling urates. These are: 1. Fat body storage and mobilization in both urate and nonurate voiding species, 2. Paternal investment, 3. Voiding urates admixed with food residues, and 4. Production of formed urate pellets (Cochran, 1981). Of these, number four has been discussed above and number three seems to be rare and will not be considered further. Numbers one and two will be explored in more detail below.
Fat Body Storage and Mobilization of Urates

The fat body is the major site of intermediary metabolism in insects, and serves as a storage organ for food reserves and, occasionally, for excretory materials (Chapman, 1982). In cockroaches, it is generally composed of three cell types: trophocytes, mycetocytes, and urocytes (Cochran, et. al., 1979). Trophocytes are the most abundant cells in the fat body. They store reserves of protein, glycogen, and lipids (Chapman, 1982). Mycetocytes in cockroaches house symbiotic bacteria which are probably involved in uric acid degradation (Cochran, 1985). Urocytes serve to store spherules of uric acid. This basic structural pattern has been found in all species (about 35) examined (Cochran, 1979).

Uric acid is primarily synthesized in the fat body (Cochran 1975). Two major pathways are involved in its synthesis; either \textit{de novo} synthesis from the catabolism of protein nitrogen or from the degradation of nucleic acids (Cochran, 1975). The \textit{de novo} synthetic pathway involves incorporation of glycine, aspartate and glutamate into the urate molecule (Chapman, 1982). In \textit{Blattella germanica}, glycine and formate are incorporated into urate in a 2:1 ratio (Engebretson, 1981), supporting the hypothesis that in insects, \textit{de novo} synthesis of urate follows the accepted pathway established for birds (Barrett and Friend, 1970).
After synthesis, urates are deposited in fat body urocytes in distinct concentric spherules, often containing radial striations (Cochran et al., 1979), or amorphous crystals (Hyatt and Marshall, 1985a, b). The size of the spherules with radial striations ranges from 6-16 μm (Tucker, 1977). These spherules have an electron dense core (Cochran et al., 1979) and are surrounded by rough endoplasmic reticulum (Martoja and Ballan-Dufraunais, 1984). These spherules and their associated membranes have been termed 'urate structural units' (Cochran et al., 1979). (Na\(^+\), K\(^+\))-ATPase activity is present in the limiting membrane of the urate crystals and in all cell membranes of the fat body (Hyatt and Marshall, 1985c). The precise composition of the urate spherules is unknown. However, uric acid is strongly bound to proteins and ninhydrin positive molecules at physiological pH (Hopkins and Lofgren, 1968) and is associated with monovalent cations (Mullins, 1981). Urate concentration in the fat body is closely related to Na\(^+\) and K\(^+\) ion concentrations (Mullins and Cochran, 1974).

Large increases in fat body urates occur during starvation (Bodenstein, 1953; Anderson and Patton, 1955; Tucker, 1977) and in cockroaches on high protein diets (Haydak, 1953; Mullins and Cochran, 1975a; Cochran et al., 1979). The accumulation of urates in the fat body of starved and semi-starved cockroaches indicates that the
insects are able to deaminate proteins and presumably utilize the carbon for energy (Haydak, 1953). Although urate content per unit weight of fat body increases during starvation, whole body urate does not significantly increase (Tucker, 1977).

On high protein diets, *Periplaneta americana* accumulate large urate stores which may be mobilized as a nitrogen reserve during times of dietary stress (Mullins and Cochran, 1975b). On 0, 2.5 or 5% protein diets formulated with dextrin, these cockroaches have shrunken abdomens with little or no urate (Haydak, 1953). When on 5% protein diets formulated with cellulose, the fat body becomes overloaded with urate stores, obscuring the cytological pattern (Cochran *et. al.*, 1979). The mycetocyte bacteria may play a major role in the degradation of urates (Cochran, 1985).

Stored urates may also function as an ion sink associated with ionic and osmotic balance (Mullins, 1974, 1981). Sodium and Potassium ions are sequestered in the fat body during dehydration and may be subsequently released during rehydration (Mullins, 1974; Hyatt and Marshall, 1977, 1985a, b). Sodium ions appear to be taken up by pre-existing urate crystals of the `amorphous granule´ type while potassium sequestration appears to occur by formation of additional spherules containing radial striations (Hyatt
and Marshall, 1985a, b). In addition, chloride ions are stored in trophocytes in crystalline form (Hyatt and Marshall, 1985a, c).

Paternal Investment of Urate and Nitrogen Transfer in Insects

Transfer of urate nitrogen to other individuals of a species may represent an evolutionary advantage over simply accumulating urates and storing them in the fat body (Cochran, 1985). The transfer of nitrogen reserves has been demonstrated in cockroaches and termites as well as other insect groups. In several species of cockroaches males have uricose glands which contain a slurry of urates (Roth and Dateo, 1964, 1965; Roth 1967, 1971). These urates are either deposited over the spermatophore at mating (Roth and Dateo, 1964, 1965; Mullins and Keil, 1980) or are consumed by the female in a post copulatory behavior (Schal and Bell, 1982). In termites, nitrogen that has been fixed by gut bacteria may be transferred from worker to soldier or from soldier to worker by trophallaxis (Bently, 1984).

Uric acid is the nitrogenous compound used by males of those cockroach species that transfer nitrogen to females (Cochran, 1985). The uricose glands of male Blattella germanica may contain about 89% uric acid on a dry weight basis (Roth and Dateo, 1964). Male B. germanica produce a slurry of urates which is deposited over the spermatophore
at mating (Roth and Dateo, 1964, 1965). The urates are found adhering to the outside of the spermatophore and are not incorporated into the sperm capsule (Roth and Dateo, 1965). These authors considered this process a means for the males to excrete urates. However, it is now known that these urates are consumed and used as a nitrogen source by the female and the developing oocytes (Mullins and Keil, 1980).

A modification of urate transfer occurs in the tropical cockroach *Xestoblatta hamata*. After copulation, females of this species feed on secretions from the male uricose glands (Schal and Bell, 1982). The feeding activity is prolonged when males have large urate stores or the female is deficient in dietary nitrogen. Field studies on *Xestoblatta hamata* indicate that females will normally feed on nitrogen deficient foods and rarely forage for uric acid. Males feed on bird and reptilian droppings which are primarily uric acid (Schal and Bell, 1982). In laboratory feeding choice studies, males feed on foods with large percentages of uric acid and high protein content. Schal and Bell (1982) suggested that evolution of the process of paternal investment of urates may be a consequence of short reproductive cycles, a relative scarcity of nitrogen-rich foods and a demand for other nutrients.
In both *Blattella germanica* and *Xestoblatta hamata* paternally derived urates are incorporated into the developing oocyte. More urates are transferred to the oocytes when females are on a low nitrogen diet (Mullins and Keil, 1980; Schal and Bell, 1982). Females on higher nitrogen diets apparently acquire less urates, thus transferring less paternally derived radiolabeled urates to the oocytes. Female *Parcoblatta fulvescens* also transfer urates, derived from injection of radiolabelled hypoxanthine, to the developing oocytes (Cochran and Mullins, 1982). Females on low dietary nitrogen (0.6%) also transferred more radiolabel to the developing oocytes than females on 3.2 or 6.7% nitrogen diets (20% and 42% protein diets). These urates gradually disappear during embryogenesis and the radiolabel is incorporated into proteins and evolved as carbon dioxide (Mullins and Keil, 1980; Cochran and Mullins, 1982).

**Structure and Function of the Insect Excretory System**

Insects tend to be found in habitats characterized by osmotic and/or ionic stress (Maddrell, 1981). This necessitates the existence of an excretory system capable of maintaining internal homeostasis by selectively eliminating excess water and ions from the hemolymph. However important
the role of osmoregulation, Wigglesworth (1972) stated that the most important function of the excretory system is the elimination of nitrogenous waste products.

The insect excretory system is generally considered to consist of the Malpighian tubule-rectum complex (fig. 2.1). The hemolymph is filtered by the Malpighian tubules, initially forming a primary urine. The flow of the primary urine is supported by an active potassium ion transport accompanied by passive flow of chloride ions and water (Cochran, 1975; Maddrell, 1971, 1981). Potassium is actively transported by pumping sites on the basal membrane of the tubule cells and passively crosses the apical membrane into the tubule lumen (O’Donnell, Maddrell and Gardiner, 1983). This creates an electrochemical and osmotic gradient which drives the flux of anions and water into the tubules.

This secretory process allows for the passive filtration of the hemolymph and removal of potentially toxic substances for which specific transport mechanisms do not exist (Maddrell, 1981). To support this passive secretion, the Malpighian tubules are frequently capable of actively transporting a wide range of compounds that the insects routinely need to eliminate (Maddrell, 1977). However, Maddrell (1981) also suggested that these active mechanisms appear not to include nitrogenous wastes. A second method by which insects can increase the rate of elimination of
Fig. 2.1 Diagrammatic summary of the excretory process in terrestrial insects (modified after Phillips, 1982).
certain compounds is by increasing the rate of secretion by the Malpighian tubules. This often occurs during diuresis in *Rhodnius prolixus* after a blood meal (Maddrell, 1963, 1964), and is facilitated by the diuretic hormone.

The primary filtrate is modified and concentrated as it passes into the hindgut. This urine is frequently mixed with food residues from the midgut (Wigglesworth, 1972). Reabsorption of water, ions and useful substances from the filtrate occurs in the anterior hindgut and rectum (Maddrell, 1981). Cochran (1975) stated that ions and water which may be excreted under one set of circumstances may be preferentially reabsorbed under other conditions to help maintain homeostasis.

Insects excrete a wide variety of nitrogenous compounds (Bursell, 1967; Cochran, 1975). The most frequently encountered, and perhaps one of the most important, of these substances is uric acid (Bursell, 1967; Cochran, 1975; Wigglesworth, 1972). Uric acid is considered to be well suited for this role mostly due to its high insolubility as a free acid or ammonium salt, allowing for the elimination of a large amount of excess nitrogen without incurring much water loss (O’Donnell et al., 1983; Wigglesworth, 1972). Other factors which make uric acid almost ideal for nitrogenous excretion are its high oxidation state, high...
nitrogen to carbon ratio and that it readily forms supersaturated solutions in physiological fluids (Cochran, 1975).

The process of uric acid excretion has been studied in the most detail in the blood-sucking Hemipteran *Rhodnius prolixus*. In 1931, Wigglesworth first observed urate crystals in the proximal third of the Malpighian tubules of this insect (1931b). It was proposed that urate secretions occurred in the distal portions of the tubules in the form of potassium urate. As the urates traveled to the proximal segment of the tubules, the potassium combined with carbon dioxide allowing the uric acid to precipitate out of solution thus forming circular concretions (Wigglesworth, 1931c). Upon analysis, these urate crystals were shown to contain mostly free uric acid and a few urate salts. When treated with alkali solutions, the urates dissolved leaving behind a distinct husk of some other material (Wigglesworth, 1931a).

In a later paper by Wigglesworth and Salpeter (1962), the histology of the Malpighian tubules of *Rhodnius* was examined in greater detail. Growing crystals of urate were noted to form around 0.1um droplets that appeared to have budded off from the microvilli of the distal tubule cells. These concretions formed between the microvilli of the brush border in the proximal third of the tubules, frequently displacing and compressing the microvilli. Although
numerous refractile elements, presumably containing phosphates or carbonates of calcium or magnesium, could be observed in the cells of Rhodnius' Malpighian tubules, solid crystals of urates have never been observed in the cells of the upper or lower tubule segments.

It had been previously demonstrated that uric acid crosses the upper walls of the Malpighian tubules entirely passively (Maddrell and Gardiner, 1974). However, the recent study by O'Donnell et. al. (1983), demonstrated that in Rhodnius prolixus, Pieris brassicae, Schistocerca gregaria, Glossina morsitans, and Tenordora sinensis, uric acid is transported against an electrochemical gradient, increasing the concentration of urate from 1.3 to over 15 times its concentration in the bathing fluid. In Rhodnius, this active transport occurred in the proximal segment of the tubule. They also reported that the urate was not changed during transport.

It is possible that uric acid transport in Parcoblatta fulvescens is a process similar to that of Rhodnius. For this to occur and produce discrete urate pellets, the pyloric valve would apparently have to retard the passage of food residues at the time the Malpighian tubules discharge their wastes (Cochran, 1973). The tubules would need to be able to recruit urates from the hemolymph at a rate rapid enough to produce a urate pellet. Unless there is a method
of regulating urate transport by the tubules, this process could interfere with osmoregulation by impeding the removal of excess salts or water when there are insufficient urates present for excretion. Alternative sites of regulation may be the in fat body or the hemolymph. In the fat body, hormonal factors could control the release of urates into the hemolymph. Transport of urates within the hemolymph may require carrier proteins which only synthesized and present during the urate excretory phase.

Cochran (1985) has suggested that the urate excretory process in *P. fulvescens* may be a highly evolved regulatory process. The movement of fecal material is apparently controlled so that the pellets either contain urates or food residues and are not a mixture. Cochran (1985) also suggested that this species is able to rapidly transport sufficient quantities of urates from the hemolymph to produce one or more urate pellets. Additional evidence for a possible regulatory role for urate excretion is that these insects can vary the amount of urate excreted by varying the number of pellets or the amount of urate contained in the pellets (Cochran, 1981).
Chapter 3
Materials and Methods

General materials and methods that were used routinely in this study are described in this section.

Rearing of *Parcoblatta fulvescens*

The insects used in this study were from a colony originally collected in Texas. They are currently in culture at Virginia Polytechnic Institute and State University. They are maintained in large glass battery jars with fiberboard spacers. Institutional-type brown paper towels are cut to fit the top of spacers and the bottom of the jars. When frequent collections are needed from a colony, the towels are omitted. A double layer of cheese cloth, held in place with a rubber band, covers the jars. Water is provided from a sponge cut to fit the bottom half of a plastic petri dish. Water is added to the sponge at two to three day intervals and, when soiled, the sponge is replaced. Dry dog food (composition in Appendix A) is placed in paper cups and is replaced as needed.

The colonies were cleaned approximately every six months. At this time, individuals from different colonies were mixed to provide for adequate genetic variability ensuring the viability of the colonies. Several colonies were infected with various parasites. The most prominent is
a antennal fungus, possibly *Herpomyces arietinus* (Roth and Willis, 1960, p. 134). To establish colonies lacking these parasites, egg cases were collected from existing colonies between April and July and placed on top of the water sponge in a clean battery jar. Egg cases collected in March and April from colonies with spring adults can produce a second generation of reproductives by early September. Later maturing colonies will normally have only one generation per year.

Individual rearing containers were used to maintain experimental animals (Cochran, 1983; Durbin, 1983). These containers (Fig 3.1) consisted of covered plastic petri dishes with a water vial placed beneath. A small glass tube with a cotton wick extending through both ends delivered distilled water by capillary action. The tube reached from the bottom of the plastic water vial through a hole in the vial's cap. A hole was either drilled or melted through the center of the petri dish bottom to allow the wick into the chamber. A harborage constructed from black paper and scotch tape was placed in the chamber. Food was prepared as described in each experiment and packed in stainless steel planchets. Fifteen of these containers fit in a specially constructed wooden rack.

For the experiments in which this apparatus was utilized, one individual was placed in each container. The racks were placed in an environmental chamber with a 14:10
Fig. 31. Cockroach rearing apparatus (from Cochran, 1983).
light:dark photoperiod. Ambient humidity ranged from 45 to 60% RH. Temperature was maintained at 27±1°C. These parameters were established to simulate mid-summer environmental conditions.

Uricase Analysis

A modified uricase analysis (Dubbs, Davis and Adams, 1956; Cochran, 1973; Mullins and Cochran, 1976) was used for all uric acid determinations. Different preparations were made depending on the determination: urate content in feces, whole body or Malpighian tubules.

Fecal pellets were weighed and, if necessary, grouped according to type before weighing. A sample of approximately 60 mg was used for non-urate pellets, and between 1.0 and 15.0 mg for urate containing pellets. The pellets were placed in a 3.0 ml glass vial with a small stirring magnet. The vials were closed with a screw on plastic cap after the addition of 2.0 ml of 0.6% Li$_2$CO$_3$ in distilled water. The vials were placed in a beaker of water heated to 60°C on a stirring hot plate. The stirring magnets inside the vials homogenized the fecal pellets as the solution was held at 60°C for 30 minutes.

Malpighian tubules, with the attached ampullae, were dissected from adult males under cold saline. All fat body and most of the tracheae were removed from the tubules.
They were rinsed rapidly in fresh saline, but not blotted since this would have caused tissue loss. Some loss of tissue may have occurred during removal, but this was estimated at less than 5% since the tubules essentially lie free in the hemocoel and are removed easily. The tubules were also placed in the 3.0 ml vials, but only 0.5 ml of Li₂CO₃ solution was added due to the small size of the tissue sample. The solution was heated and homogenized as described for fecal pellets.

Insects to be used for whole body analysis were collected and frozen prior to preparation. The insects were weighed to determine wet weight then dried to constant weight in an oven set at 80°C. The carcasses were ground with a mortar and pestle and 25 mg samples were used for extraction as described above.

After extraction, the homogenates were centrifuged in a Sorval refrigerated centrifuge set at 5,000 rpm (2,000 x g) for ten minutes. Since capped vials were used during extraction, loss of the solution due to evaporation was minimal and the original amount of Li₂CO₃ solution added could be used to determine the volume of the supernatant. An aliquot of the supernatant was used for the analysis of fecal pellets and whole body urates, while the entire sample was used for Malpighian tubule analysis due to the small sample size.
Glycine buffer was used for the uricase assay. This buffer was made from 7.5 g glycine in 800 ml of distilled water. Approximately 8.0 ml of 5N NaOH was added to adjust the pH to 9.4. The buffer was diluted to 1.0 liter and the pH rechecked and adjusted with NaOH if necessary. Uricase enzyme solution was prepared before each assay. Hog liver type IV uricase, obtained from Sigma Chemical Corp., was used to make a 2.0 mg protein/ml glycine buffer enzyme solution.

Before assaying, a 10-200 ul aliquot of either a fecal pellet or whole body extract was added to 4.8 ml of glycine buffer and the absorption checked at 292 nm against a glycine buffer blank. The aliquot size was initially estimated from the expected concentration of uric acid in the sample. If the absorption was below 0.3 or above 0.7, a second dilution of the sample was made to reach the desired initial absorption. To initiate the reaction 200 ul of uricase enzyme solution was added, the reaction mixture was vortexed, and an initial absorption reading taken. Absorption was checked every 30 minutes until no change in absorption was measured. Between readings, the test samples were incubated at 37°C in a hot water bath. Normally, within two hours the reaction was complete.
The amount of uric acid in the initial sample was determined by taking the change in absorption, total volume, Beer's Law and the extinction coefficient determined from a standard curve to calculate the amount of uric acid in the test sample. The total urate content was calculated by dividing the amount of uric acid in the aliquot by the fraction of the whole sample that the aliquot represented.

Some modifications of this procedure were required to determine urate content in the Malpighian tubules. These were necessary due to the small size of the sample. The total volume of the supernatant after centrifugation (0.5 ml) was used for one assay. The supernatant was placed in a quartz cuvette. To this volume, 0.30 ml of glycine buffer and 0.20 ml of enzyme solution were added. Immediately after the reaction mixture was shaken by inverting the cuvette three times, initial absorption was measured. The cuvettes were left in the spectrophotometer and absorption recorded every 10 minutes until the end-point was reached. Urate content in the sample was calculated from the change in absorption and total volume as described above.

Diet Preparation

The preparation and composition of the various diets used in this study are given in their respective chapters.
Chapter 4

Uric Acid Excretion by Last Instar Juvenile

Parcoblatta fulvescens (Dictyoptera: Blattellidae)

Abstract

The pattern of uric acid excretion during the last juvenile instar of Parcoblatta fulvescens and its correlations to diet, sex and food consumption were investigated. Newly ecdysed individuals were collected from the rearing colonies, sexed and placed on either a 15, 25 or 42% protein diet. Food consumption and fecal pellet formation were monitored daily until the imaginal molt. Uric acid content of the formed urate pellets was determined with a standard uricase assay. The patterns of food consumption, urate and non-urate pellet formation show a skewed distribution with peak feeding occurring on day six and peak voiding of both pellet types on day seven of a 17.0 ± 2.0 (SD) day ecdysial cycle. Sex did not significantly influence the amount of urate voided, but did affect the amount of food consumed and non-urate pellet output. The level of protein in the diet was found to be the most significant factor (p<0.0001) in determining the amount of uric acid voided.
Introduction

The wood cockroach *Parcoblatta fulvescens* is one of the few species of cockroaches known to void uric acid. They package urates in discrete formed pellets (Cochran, 1973) containing up to 70% uric acid by weight (Cochran, 1976, 1981). The level of dietary nitrogen relative to metabolic demand is one of the controlling factors in urate excretion. Adult females and large nymphs will not void urates unless dietary nitrogen is above 3.0% and 1.5%, respectively (Cochran and Mullins, 1982). Cochran (1985) refers to this as a "break-even point" for urate voiding, where dietary nitrogen or protein is just adequate to meet the nitrogen demand of the individual.

*Parcoblatta fulvescens* is also capable of storing urates in the fat body (Cochran, 1979). In the cockroach fat body, stored urates may function as an ion sink (Mullins and Cochran, 1974; Tucker, 1977; Hyatt and Marshall, 1977, 1985a, b, c) or nitrogen reserve (Haydak, 1953; McEnroe and Forgash, 1956; Mullins, 1971; Mullins and Cochran, 1975b). The excretion of urates and their central role in cockroach physiology was recently reviewed (Cochran, 1985).

This study is part of an ongoing investigation of the physiology of urate excretion in *Parcoblatta fulvescens* and the possible biological significance of the formed urate pellets. The purpose of this study is to show the pattern
of food consumption and fecal pellet output in relation to
the ecdysial cycle, and to demonstrate the relationship
between urate voiding and the amount of protein consumed
during the ecdysial cycle.

Materials and Methods

The cockroaches used were from a colony collected in
Texas and maintained in laboratory culture at Virginia
Polytechnic Institute and State University. Newly ecdysed
large nymphal Parcoblatta fulvescens were collected from the
end of April to late June, when the maturation rate appeared
to be most rapid. This experiment was originally attempted
in early winter, but during that time this species
has a prolonged developmental period, which has also been
observed by Rau (1940).

Each nymph was sexed and placed in individual rearing
chambers described previously (Durbin, 1983; Cochran, 1983). Briefly, these chambers consisted of a plastic petri dish
with a small hole in the center of the bottom. Through
this hole, a small glass rod with a cotton wick delivered
water from a plastic water vial placed under the petri dish.
Fifteen of these chambers fit on a custom made wooden rack.
These racks were kept in a chamber with controlled
temperature (27±1°C) and a 14:12 light:dark photoperiod.
Humidity ranged from 45-60% RH.
Twelve to fifteen individuals of each sex were placed on one of three diets consisting of 15, 25 or 42% protein. The 25% protein diet was composed entirely of ground dog food, which contains 25% crude protein fiber. The 42% diet consisted of 7:2 ground dog food to casein protein. A modified Haydak (1953) 5% protein, dextrose diet (86% dextrose, 10% brewers yeast extract and 4% Hawk Osser salt mixture #3) was mixed with an equal weight of dog food to provide the 15% diet. The diets were packed in stainless steel 1 x 5/6 in. planchets and steamed for 10 min. to produce a hardened cake (Mullins and Cochran, 1973). This allowed for the insects to feed without displacing the diets.

The planchets were removed daily and weighed on a Mettler balance (Model B; capacity 0-200g; precision ± 0.03mg; readability 0.1mg) to monitor food consumption. Five control planchets for each diet were also weighed daily to provide a correction factor for humidity changes. Fecal pellets were collected from each chamber, grouped according to type (urate or non-urate) and weighed on a Mettler ME-100 digital balance. The weights of the feces were not corrected for wet weight.

Prior to uricase analysis, the urate and non-urate fecal pellets were pooled according to type for the entire instar of each individual. In addition, the non-urate pellets were again pooled for each sex on each diet. The
pellets were placed in a 3.0 ml glass vial with a plastic screw-on cap. A small stirring magnet and 2.0 ml of 0.6% Li$_2$CO$_3$ were added to the vials. The vials were placed in a beaker of water on a stirring hot plate and homogenized with the stirring magnet while being held at 60°C for 30 min. The mixture was centrifuged in a Sorval refrigerated centrifuge set at 5,000 rpm (2,000 x g) for 10 min. A 10 to 250ul sample of the supernatant was used for the uricase analysis modified from Dubbs et al. (1956). The sample size was estimated from the weight and type of the fecal pellets and the initial absorption of the reaction mixture. Hog liver type V uricase was obtained from Sigma Chemical Co. Quantitation of uric acid was achieved by calculation based on the change in absorption of the unknowns at 292nm and the extinction coefficient of uric acid determined from a standard curve.

Data collection began the first day after the molt and continued daily until the imaginal molt. In some cases, this required monitoring individuals over two ecdysial cycles. Since the length of the ecdysial cycle was not the same for all individuals, the data were aligned according to the days of the last juvenile and the imaginal molts. Differences in food consumption and urate pellet excretion were determined with a general linear models procedure and the relationship between the amounts of protein consumed and uric acid excreted was described with linear regression.
Results

The number of last instar juveniles of each sex and placed on either the 15, 25 or 42% protein diets that completed the required ecdysial cycle are given in Table 4.1. These numbers were used for all calculations. The patterns of food consumption and fecal pellet production over the preimaginal ecdysial cycle are shown in Fig. 4.1 a. This graph is a composite of both sexes on all three diets and gives the average daily consumption of all individuals. It should be noted that not all individuals feed or excrete on any given day and the percentages are not given. All three patterns are similar in that they show a skewed distribution toward the first half of the ecdysial cycle. Peak feeding occurred on day six and peak voiding of non-urate pellets occurred on day seven of a 17 ± 2.0 (SD) ecdysial cycle. Peak voiding of urate pellets occurred between days six and eight. It should be noted that urate pellet excretion by one individual (Fig. 4.1 b,c) does not always occur on consecutive days.

Further analysis of the patterns for food consumption, urate and non-urate pellet, and total excreta voided showed some differences for each dietary group (Fig. 4.2 a,b,c,d). These graphs show average consumption and voiding. Not all individuals feed or excrete on a given day. These
Table 4.1. Number of last instar juveniles of either sex within each dietary group used in this study.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>15% Protein</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>25% Protein</td>
<td>10</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>42% Protein</td>
<td>11</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>32</strong></td>
<td><strong>62</strong></td>
</tr>
</tbody>
</table>
Fig. 4.1 a. Food consumption (⋯⋯⋯⋯), urate pellet (⋯⋯⋯⋯), and normal pellet (⋯⋯⋯⋯) excretion during the last juvenile instar. Composite graph for all individuals (N=62).
Fig. 4.1 b. Food consumption (●—●), urate pellet (●—●), and normal pellet (●—●) excretion during the last juvenile instar by one female on the 25% protein diet.
Fig. 4.1 c. Food consumption (—.), urate pellet (— — —), and normal pellet (— — —) excretion during the last juvenile instar by one female on the 42% diet.
Fig. 4.2 a. Daily food consumption during the last juvenile instar.

- - - •, 15% protein diet; •—•, 25% protein; •—•, 42% protein.
Fig. 4.2 c. Daily normal fecal pellet excretion during the last juvenile instar. •—•, 15% protein diet; — ••, 25% protein; — — •, 42% protein.
Fig. 4.2 The daily urate pellet excretion during the last juvenile instar. •—•, 15% protein diet; •—•, 25% protein; •—•, 42% protein.
individuals were, however, included in the calculations of average consumption and excretion. The patterns for food consumption, total and normal-fecal pellet excretion show the skewed distribution toward the first half of the preimaginal instar as described above, with little effect due to diet evident. The most striking difference is in the pattern for urate pellet voiding (Fig. 4.2 d). All individuals on the 42% diet produced urate pellets. One female on the 25% diet failed to excrete urates and one male on the 15% diet produced a single fecal pellet which contained uric acid. The amount of protein in the diet was the most significant factor (p<0.0001) in determining the amount of uric acid voided.

The days of onset and cessation of feeding and fecal pellet production are presented in Table 4.2. On average, 50% of the individuals of a given sex in any of the dietary groups will begin to feed or excrete before the days given in the graph, and 50% of the individuals will continue to void fecal pellets or consume food after the average day of cessation of these activities. The delay between the onset of feeding and the production of urate pellets averages 3.2 days, while urate pellet excretion ceases 4.2 days before feeding.
Table 4.2. Days of onset and cessation of feeding, normal fecal pellet and urate pellet voiding during the preimaginal ecdysial cycle of *Parcoblatta fulvescens*. Values are expressed as mean (in days) ± standard error of the mean.

<table>
<thead>
<tr>
<th>Diet and Sex</th>
<th>Days of Onset for Feeding</th>
<th>Normal pellet voiding</th>
<th>Urate Voiding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>2.6 ± 0.4</td>
<td>1.9 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td>2.4 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>4.0 (N=1)</td>
</tr>
<tr>
<td>25% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>1.9 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td>Males</td>
<td>2.9 ± 0.7</td>
<td>1.6 ± 0.2</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>42% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>2.0 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Males</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>5.5 ± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet and Sex</th>
<th>Days of Cessation for Feeding</th>
<th>Normal pellet voiding</th>
<th>Urate Voiding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>14.2 ± 0.7</td>
<td>14.6 ± 0.7</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td>16.3 ± 1.0</td>
<td>16.3 ± 1.0</td>
<td>4.0 (N=1)</td>
</tr>
<tr>
<td>25% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>13.9 ± 1.4</td>
<td>14.1 ± 0.7</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>Males</td>
<td>13.4 ± 0.5</td>
<td>14.1 ± 0.5</td>
<td>10.5 ± 0.8</td>
</tr>
<tr>
<td>42% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>12.7 ± 0.7</td>
<td>12.8 ± 0.5</td>
<td>9.7 ± 0.4</td>
</tr>
<tr>
<td>Males</td>
<td>13.7 ± 0.5</td>
<td>13.7 ± 0.5</td>
<td>10.4 ± 0.5</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>14.0 ± 0.4</td>
<td>14.2 ± 0.3</td>
<td>9.8 ± 0.4</td>
</tr>
</tbody>
</table>
Upon examination of the effect of sex on food consumption and voiding, two trends appear (Table 4.3). First, females consumed more food and produced more excreta, than males. Second, females on the 42% diet excreted more uric acid than males, while the reverse was true for the 25% diet. In addition, females typically had shorter ecdysial cycles than males and as the level of protein in the diet increased, the length of the ecdysial cycle became shorter.

The relationship between mg protein consumed and mg uric acid excreted during the last juvenile instar is shown in Fig. 4.3. Although the linear regression correlation coefficients are not as high as would be desired, the Pearson product-moment shows a high probability (p<0.0001) for a positive correlation between protein consumption and urate excretion. The low correlation coefficients may be in part due to differences in nitrogen balance between individuals at the onset of the experiment and to individual variation. The 95% confidence limits (not shown) on the lines are mutually exclusive, suggesting a difference in metabolic demand for protein in males and females during the preimaginal molt. The x-axis intercepts indicate the average amount of protein required for the preimaginal instar. Above these protein levels, excretion of urates may be expected.
Table 4.3. Effects of diet and sex on length of ecdysial cycle, food consumption and fecal pellet voiding during the last juvenile instar of *Parcoblatta fulvescens*. Values are expressed as mean ± standard error of the mean.

<table>
<thead>
<tr>
<th>Diet and Sex</th>
<th>Length of Ecdysial Cycle (days)</th>
<th>Food Consumed (mg)</th>
<th>Protein Consumed (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>17.0 ± 0.7</td>
<td>117.2 ± 11.8</td>
<td>17.6 ± 1.8</td>
</tr>
<tr>
<td>Males</td>
<td>18.9 ± 1.0</td>
<td>84.9 ± 1.8</td>
<td>12.7 ± 0.9</td>
</tr>
<tr>
<td>25% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>16.8 ± 0.6</td>
<td>87.5 ± 7.4</td>
<td>21.9 ± 1.9</td>
</tr>
<tr>
<td>Males</td>
<td>17.4 ± 0.4</td>
<td>81.2 ± 0.4</td>
<td>20.3 ± 1.9</td>
</tr>
<tr>
<td>42% Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>15.5 ± 0.4</td>
<td>100.2 ± 4.9</td>
<td>42.1 ± 2.0</td>
</tr>
<tr>
<td>Males</td>
<td>16.9 ± 0.4</td>
<td>63.7 ± 5.2</td>
<td>26.7 ± 2.2</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>17.0 ± 0.3</td>
<td>88.0 ± 3.5</td>
<td>24.2 ± 1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet and Sex</th>
<th>mg Normal Pellets Excreted</th>
<th>mg Urate Pellets Excreted</th>
<th>mg Uric Acid Excreted</th>
<th>Percentage Uric Acid in Urate Pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>15% Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>17.2 ± 2.0</td>
<td>0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td>17.1 ± 2.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>59.4 (N=1)</td>
</tr>
<tr>
<td>25% Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>18.9 ± 1.5</td>
<td>3.0 ± 0.6</td>
<td>1.4 ± 0.3</td>
<td>45.3 ± 6.4</td>
</tr>
<tr>
<td>Males</td>
<td>15.0 ± 1.2</td>
<td>4.5 ± 0.8</td>
<td>2.6 ± 0.5</td>
<td>57.8 ± 1.8</td>
</tr>
<tr>
<td>42% Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>13.7 ± 0.8</td>
<td>12.4 ± 1.2</td>
<td>6.8 ± 0.6</td>
<td>54.7 ± 2.3</td>
</tr>
<tr>
<td>Males</td>
<td>10.3 ± 0.6</td>
<td>8.1 ± 0.9</td>
<td>4.1 ± 0.4</td>
<td>50.4 ± 2.7</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>16.6 ± 0.9</td>
<td>5.1 ± 0.6</td>
<td>2.7 ± 0.3</td>
<td>53.5 ± 1.4*</td>
</tr>
</tbody>
</table>

* 15% protein diet omitted from calculation to avoid bias in the calculation of % uric acid in urate pellets from missing values.
Fig. 4.3. Relationship between protein consumption and uric acid excretion during the last juvenile instar. ▲ Males, $r^2 = 0.78$; ○ Females, $r^2 = 0.86$. 

MC URIC ACID EXCRETED VS. MG URIC ACID CONSUMED
Discussion

The impact of dietary nitrogen levels on uric acid voiding by juveniles and reproductive females has been previously demonstrated (Cochran, 1981; Cochran and Mullins, 1982). These authors showed that as the level of dietary nitrogen increased above 3.0% and 1.5% for adult females and large nymphs, respectively, the amount of uric acid excreted also increased. The results of this study not only support this finding but also show a closer relationship between protein consumption and urate voiding during the preimaginal ecdysial cycle. In addition, a difference between male and female preimaginal juveniles in protein demand is evident.

Cochran (1985) had defined a "break-even point" with respect to urate voiding as the level of protein in the diet which is just adequate to meet the nitrogen demands of reproducing females. Above this threshold level, urate voiding may be expected. A redefinition of the break-even point may be suggested as the amount of protein consumed which is just adequate to meet the nitrogen demands of individuals during a specific developmental stage. Below this level, prolonged or incomplete development is possible.

The patterns of food consumption and fecal pellet voiding also suggest a relationship between metabolism of protein and urate excretion. The onset of urate pellet excretion occurs after the onset of feeding and non-urate
pellet voiding (Table 4.2) suggesting that a certain amount of time for metabolism and possibly for a build-up of stored urates is required before the onset of urate excretion. An additional factor requiring further study is the possibility for a threshold level of urates in the fat body below which urates will be stored, if excess nitrogen is available, and above which urates will be mobilized for excretion.

Although the amount of protein consumed is one of the controlling factors in urate voiding for *Parcoblatta fulvescens*, the exact mechanism by which urate excretion is regulated is unknown. Since urate voiding may represent a regulatory mechanism by which nitrogen balance is maintained, there may be some hormonal involvement in urate excretion. This could occur at several sites within the insect including the fat body, hemolymph or Malpighian tubules. In the fat body, there may be hormonal signals causing either urate storage or urate release into the hemolymph. Urate excretion may also be controlled by the presence or absence of urate carrier proteins or by the presence or absence of ability of the Malpighian tubules to secrete uric acid. Further study of the means by which *Parcoblatta fulvescens* and other urate-voiding cockroaches regulate uric acid excretion is warranted.
Chapter 5
Diet Selection During the Oothecal Cycle
of Parcoblatta fulvescens

Abstract

The ability to selectively feed on diets containing primarily protein, carbohydrate or cellulose was investigated. Adult female Parcoblatta fulvescens that were given the opportunity to mate were maintained for three oothecal cycles on a choice of three different diets containing either 42% protein, 86% carbohydrate and 5% protein or 86% cellulose and 5% protein. Food consumption on each of these diets was monitored daily for the second two egg case cycles to determine if changes in dietary preference occurred during the cycle. For each diet a separate pattern for consumption was evident. Peak feeding on the protein, dextrose and cellulose diets occurred on the day of egg-case drop, one day following egg-case drop and two days before the appearance of the egg case, respectively. When given this dietary choice, females did not excrete uric acid.
Introduction

The wood cockroach *Parcoblatta fulvescens* is one of the few species of cockroaches known to void uric acid (Cochran, 1972; 1979). Dietary nitrogen has been determined to be one of the controlling factors in the excretion of urates (Cochran, 1981). The threshold value for urate excretion by adult females was determined to be between 20 and 25% protein. Other physiological parameters regulating this process, especially those related to diet, are unknown.

The native diet of *P. fulvescens* has not been determined. Examination of gut contents of five *Parcoblatta* sp. nymphs by Cochran and Mullins (1982) showed that this species may feed on decaying plant debris that is low in nitrogen and animal and fungal material that are higher in nitrogen than the dog chow used in laboratory rearing. Other species of the genus *Parcoblatta* have been observed to feed on mushrooms, mammalian and bird feces, sap from wounded trees and some plant materials (Gorton, 1980). Field studies on a tropical cockroach, *Xestoblatta hamata*, indicate that females feed on nitrogen deficient foods while males will feed on reptilian and bird droppings (Schal and Bell, 1982). These data suggest that cockroaches may be capable of selecting their diet relative to nitrogen demand.
This experiment was initiated after the observation that adult males which had been maintained on a low nitrogen high carbohydrate diet for several months initiated feeding on a high nitrogen food source within fifteen minutes after it was offered.

Four major objectives were involved in this experiment: 1. to determine whether mated adult female Parcoblatta fulvescens are capable of selecting various dietary sources during the oothecal cycle and if any sequential changes in the amount of protein, carbohydrate or cellulose consumed occur, 2. to give an indication of the ratio of protein to carbohydrate (nitrogen to energy) that the females may scavenge for in their native environment, 3. to determine if urate is excreted when females are given a dietary choice, and 4. to determine the amount of uric acid stored when adult females are given a dietary choice.

Materials and Methods

Twenty-two adult female Parcoblatta fulvescens, less than 60 days from the imaginal molt and carrying normal looking egg cases, were collected from the rearing colonies at VPI & SU. Each female was placed in an individual rearing chamber that provided water and a harborage as described previously (Cochran, 1983; Durbin, 1983). Three diets
containing either 42% protein, 86% cellulose or 86% dextrose (modified after Haydak, 1953) were provided to each female. These diets were formulated as follows: 1. 78% ground dog food and 22% casein protein, 2. 86% chromatographic grade cellulose, 10% brewers yeast extract and 4% Hawk Oser #3 salt mixture, or 3. 86% anhydrous reagent grade dextrose (d-glucose), 10% brewers yeast extract and 4% Hawk Osser salt mix #3. The brewers yeast extract contained 50% protein, thus the final protein content of the cellulose and dextrose diets was 5%. The diets were packed into stainless steel planchets to allow for easy removal of the food from the rearing chamber and to help prevent spillage during feeding. The rearing vessels were maintained in an environmental chamber at 27±1 °C with a 14:10 light:dark photoperiod. Humidity ranged from 45-60% RH.

The females were equilibrated on the diets for the first egg case cycle after placement in the chambers. The food dishes were weighed daily from the fifth day after the females were collected to the day of the fourth egg case drop to determine food consumption. Five control dishes for each diet were also weighed daily to provide a correction factor for changes in weight due to fluctuations in humidity. For analysis, the data were aligned according to the major events of the oothecal cycle: The first day that the egg case was visible and the day of egg-case drop.
Fecal pellets produced between the appearance of the second egg case and the fourth egg case drop were collected and assayed for uric acid using a modified uricase assay (Cochran, 1973; Dubbs et al., 1965; Mullins and Cochran, 1976). In addition, five females from this experiment were frozen within three days of the last egg-case drop. The cockroach bodies were dried, extracted in Li₂CO₃ and analyzed for urate using the uricase analysis.

Results

Of the 22 individuals initially used in the experiment, 15 produced the necessary two egg cases. Of the ones that did not complete the experiment, three died after, and one before the end the first egg case cycle of unknown causes. The remainder did not produce the terminal egg case of the experiment.

The patterns of consumption of the protein, dextrose and cellulose diets are shown in Fig. 5.1. Total daily consumption of all diets shows two peak feeding periods on days four and six and low food consumption when the females are carrying egg cases. Dextrose diet consumption has a very similar pattern, but low food consumption begins the day before the appearance of the egg case. Protein diet consumption peaked on the day of egg-case drop and gradually
Day of the Reproductive Cycle of Adult Female *Parcoblatta fulvescens*

Fig. 5.1 Food consumption by adult female *Parcoblatta fulvescens* over the reproductive cycle when given a dietary choice. Legend: -- --, 5% protein-cellulose diet; -----, 5% protein-dextrose diet; ---, 42% protein diet. EC, first day egg-case is visible; ECD, day of egg-case drop.
declined until the succeeding egg-case drop. Consumption of cellulose was not expected at the onset of the experiment since this diet contains little nutrient value. Especially remarkable was the increase in cellulose consumption, peaking on day 6, during the second half of the oothecal cycle.

The values for average daily consumption and total daily consumption of each diet are given in Table 5.1. For comparison, values obtained for consumption of each diet by females in a non-choice situation are also given. The ratio of carbohydrate to protein diet consumed by females given the dietary choice was 1.48. This value corresponds to a diet composed of 16.4% protein and 38.6% utilizable carbohydrate; a carbohydrate to protein ratio of 2.35. If the value for cellulose diet consumption is omitted, due to its low nutritional value, a diet corresponding to 40.7% protein and 50.8% carbohydrate is obtained (carbohydrate to protein ratio=1.25). When the amount of lipid (8.0%) contained in the dog food used to formulate the 42% protein diet is taken into consideration, a 2.47 ratio of carbon to nitrogen containing compounds is obtained.

The average lengths of the cycle and the average terms for carrying the egg case are given in Table 5.2. The values for females given the dietary choice agree, reasonably well with previously recorded values (Cochran,
Table 5.1 Average daily consumption and total consumption of cellulose, dextrose and protein diets by female Parcoblatta fulvescens during the reproductive cycle given a dietary choice compared with females on the same diets without having a choice. The values given for consumption by females on the cellulose diet alone are based on only one female due to the high mortality of females on this diet. Values are given as means ± standard error of the mean.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Daily consumption</th>
<th>Total consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With Dietary Choice (N=30)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose*</td>
<td>4.1 ± 0.4</td>
<td>35.1 ± 7.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>7.6 ± 0.4</td>
<td>64.3 ± 4.3</td>
</tr>
<tr>
<td>Protein</td>
<td>5.1 ± 0.3</td>
<td>43.5 ± 3.8</td>
</tr>
<tr>
<td>Total</td>
<td>16.8 ± 0.8</td>
<td>142.9 ± 8.6</td>
</tr>
<tr>
<td><strong>Without dietary Choice</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose* (N=1)</td>
<td>22.4</td>
<td>245.9</td>
</tr>
<tr>
<td>Dextrose* (N=5)</td>
<td>11.9 ± 2.9</td>
<td>110.4 ± 26.4</td>
</tr>
<tr>
<td>Protein (N=5)</td>
<td>10.9 ± 2.2</td>
<td>77.4 ± 17.3</td>
</tr>
</tbody>
</table>

* Both the cellulose and dextrose diets contain 5% protein
Table 5.2 Length of the oothecal cycle for female Parcoblatta fulvescens given a dietary choice and compared with females not given a dietary choice. Values are given as mean ± one standard error of the mean.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Number</th>
<th>Length of oothecal cycle</th>
<th>Term for carrying egg-case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choice</td>
<td>30</td>
<td>8.0 ± 0.1</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Dextrose*</td>
<td>5</td>
<td>11.0 ± 1.6</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Protein</td>
<td>5</td>
<td>7.0 ± 0.8</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Cellulose*</td>
<td>1</td>
<td>11.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Both the dextrose and cellulose diets contain 5% protein
personal communication). For comparison, the values for the length of the oothecal cycle for the choice situation and each diet alone (no choice) are given in Table 5.2. The length of the oothecal cycle for females on the cellulose diet is misleading since most females cannot produce a second egg case.

The amount of stored urate nitrogen within five of the females given a dietary choice are given in Table 5.3. Stored urate nitrogen averaged $0.21 \pm 0.07$ umoles/mg. The average dry weight of these females was $88.9 \pm 10.1$ mg. No uric acid was detected in the fecal pellets produced by the females during the experiment. These data indicate that the females were able to regulate the amount of protein consumed relative to their metabolic demand.

Discussion

The results presented in this chapter show that reproductive female *Parcoblatta fulvescens* are capable of selectively feeding on food sources with different protein, carbohydrate and cellulose content, and given this food choice do not excrete uric acid. Previous work has shown that the level of protein in the diet and the amount of protein consumed are the major controlling factors in uric
Table 5.3. The amount of uric acid stored within five *Parcoblatta fulvescens* females given a dietary choice.

<table>
<thead>
<tr>
<th>Female number</th>
<th>Dry weight (mg)</th>
<th>Stored uric acid nitrogen (umoles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.4</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>90.4</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>119.3</td>
<td>0.35</td>
</tr>
<tr>
<td>4</td>
<td>60.8</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>74.5</td>
<td>0.12</td>
</tr>
<tr>
<td>mean±S.E.M.</td>
<td>88.9±10.1</td>
<td>0.21±0.07</td>
</tr>
</tbody>
</table>
acid excretion in *Parcoblatta fulvescens* when the individuals are not given a food choice (Cochran, 1981; Cochran and Mullins, 1982).

Cochran (1981) suggested that changes in food preference or availability in nature may modulate nitrogen excretion. This work supports this suggestion, in that changes in food preference can occur during the oothecal cycle (Fig. 5.1) and that reproductive females are apparently capable of regulating the amount of protein consumed to a level which meets their nutrient requirements. The shifts in dietary preference are not yet understood, but may represent a shift in nitrogen and carbohydrate demand during the oothecal cycle.

Consumption of the cellulose diet was not expected at the onset of this experiment. This diet contains 5% protein and essential vitamins and minerals, but has little other nutritional value. It may be possible that gut microbes and symbionts can degrade the cellulose, offering additional utilizable carbohydrate to the individual. This does not, however, account for the increased cellulose diet consumption during the latter part of the cycle (Fig. 5.1).

The actual percentage protein consumed from the three diets (16.4%) is lower than the "break-even point" of 20-25% casein protein for reproducing female *Parcoblatta fulvescens* (Cochran, 1985), while the value for percentage protein when the cellulose diet is omitted (40.7%) is much higher. This
may be due to the availability of carbohydrate in the diet. If a carbohydrate source is freely available, the insects may require less protein as an energy source. The protein intake may then be used solely to meet the nitrogen demand of females. Further analysis of the actual amount of protein consumed from diets with differing protein and carbohydrate levels may help explain this situation.

The values obtained for whole body urate nitrogen suggest that the females are able to closely regulate the amount of uric acid stored in the fat body. The amount obtained (0.21±0.07 umoles/mg, n=5) is lower than that for females on a 20% casein protein diet of 0.37±0.18 umoles/mg (n=4; Cochran and Mullins, 1982). Three factors could have resulted in this difference: 1. Uric acid may have degraded to some extent during storage of the bodies, 2. Females on the choice diet did not excrete urates, therefore the hindguts did not contain this compound, and 3. the lipid content of the females may be higher than that of females on a restricted protein diet. The second of these alternatives is perhaps the most significant since the hindgut may hold large quantities of urate.

The exact food sources for wood cockroaches are not entirely known. The food choice given in this experiment may represent an almost ideal situation for reproductive female Parcoblatta fulvescens. It is unlikely that protein
and carbohydrate sources will be freely available to each individual. Therefore, depending on food availability, excess carbohydrate or nitrogen may be consumed. This would require storage of carbohydrates as lipids or glycogen and mobilization of urates for nitrogen when excess carbohydrates and little protein is available and storage or excretion of urates when carbohydrate is restricted and high nitrogen food sources are available.
Chapter 6

Urate Pellet Consumption by Adult Female Parcoblatta fulvescens (Saussure and Zehntner) (Dictyoptera: Blattellidae).

Abstract

The consumption of uric acid-containing fecal pellets by reproductive females Parcoblatta fulvescens was examined in relation to dietary protein and carbohydrate. Urate pellet consumption increases with decreasing protein and increasing carbohydrate levels. These results suggest that the urate-containing pellets actually do serve to transfer nitrogen reserves among individuals.
Introduction

The transfer of urates between individuals as a nitrogen reserve has been previously observed in two species of cockroaches. *Blattella germanica* and *Xestoblatta hamata* males both transfer urates to the females at mating. Male *Blattella germanica* uricose glands produce a slurry of urates which is deposited over the spermatophore at mating (Roth and Dateo, 1964, 1965). These urates are consumed and apparently used as a nitrogen source by the female and the developing oocytes (Mullins and Keil, 1980). After copulation, female *Xestoblatta hamata* feed on the secretions from the male's uricose glands. This activity is prolonged when males have large urate stores (Schal and Bell, 1982). Schal and Bell also demonstrated that these paternally derived urates are transferred to the oocytes.

Although male *Parcoblatta fulvescens* do not have uricose glands, this species is one of the few species of cockroaches that are known to void uric acid as a nitrogenous excretory product (Cochran, 1973, 1976, 1979, 1981). These cockroaches excrete urates in compact white fecal pellets that are distinct from the normal fecal pellets. It has been suggested that these pellets may serve as a means to transfer nitrogen between individuals of *Parcoblatta* after the pellets have been subject to the weathering process (Cochran, 1979, 1985).
The purpose of this study is to examine this hypothesis. This study was initiated after it was observed that four virgin, egg-case producing females that had been maintained on a low nitrogen, high carbohydrate diet consumed unmodified urate pellets. This was an unexpected finding. Experimentation has revealed that a carbohydrate source is very important in urate-pellet consumption.

Materials and Methods

Adult female *Parcoblatta fulvescens* less than 60 days from the imaginal molt and carrying normal looking ootheca were selected from the rearing colonies maintained in the laboratory. These colonies were originally obtained from Texas. They are maintained in glass battery jars with water and dog chow *ad libitum*. The females were placed in individual rearing containers that provided water and a harborage (Cochran, 1983; Durbin, 1983). These containers were placed in an environmental chamber with a 14:10 light:dark photoperiod and temperature set at 27 ± 1 °C. Humidity ranged from 45-60% RH.

Five females were placed on each of a series of graded protein, cellulose and carbohydrate diets (Table 6.1). These females were replaced when any died or escaped to provide the necessary five females on each diet. The
Table 6.1. Composition of diets used to study urate pellet consumption. C= cellulose, D= dextrose, DC= 50-50 dextrose-cellulose. P= protein. The 5% protein-dextrose diet is from Haydak (1953).

### Basic diet composition:

<table>
<thead>
<tr>
<th></th>
<th>Dextrose</th>
<th>Cellulose</th>
<th>Hawk Oser Salt Mix #3</th>
<th>Brewer's Yeast Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% P-D</td>
<td>86%</td>
<td>0%</td>
<td>4%</td>
<td>10%</td>
</tr>
<tr>
<td>5% P-C</td>
<td>0%</td>
<td>86%</td>
<td>4%</td>
<td>10%</td>
</tr>
<tr>
<td>5% P-DC</td>
<td>43%</td>
<td>43%</td>
<td>4%</td>
<td>10%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Milled dog chow</th>
<th>Casein Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% P</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>34.4% P</td>
<td>87.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>42% P</td>
<td>77.8%</td>
<td>22.2%</td>
</tr>
</tbody>
</table>

### Graded Protein Diets:

<table>
<thead>
<tr>
<th></th>
<th>25% P</th>
<th>5% P-D</th>
<th>5% P-C</th>
<th>5% P-DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% P-D</td>
<td>25%</td>
<td>75%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>10% P-C</td>
<td>25%</td>
<td>0%</td>
<td>75%</td>
<td>0%</td>
</tr>
<tr>
<td>10% P-DC</td>
<td>25%</td>
<td>0%</td>
<td>0%</td>
<td>75%</td>
</tr>
<tr>
<td>15% P-D</td>
<td>50%</td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>15% P-C</td>
<td>50%</td>
<td>0%</td>
<td>50%</td>
<td>0%</td>
</tr>
<tr>
<td>15% P-DC</td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
<td>50%</td>
</tr>
<tr>
<td>20% P-D</td>
<td>75%</td>
<td>25%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>20% P-C</td>
<td>75%</td>
<td>0%</td>
<td>25%</td>
<td>0%</td>
</tr>
<tr>
<td>20% P-DC</td>
<td>75%</td>
<td>0%</td>
<td>0%</td>
<td>25%</td>
</tr>
</tbody>
</table>
cellulose and carbohydrate diets were modified after Haydak (1953). These diets were packed in stainless steel planchets. All the diets except the 5% dextrose, 5% dextrose:cellulose, and 10% dextrose were steamed for ten minutes to form a hardened cake that the females could feed on but not displace. The remaining diets were not steamed to prevent the formation of a 'caramel'.

The females were allowed to equilibrate on the diet for one oothecal cycle. On the days that the second, third, and fourth egg cases were dropped, the weight of the food was recorded and all fecal pellets removed. In the 24 hours following the third egg case drop, a known weight of urate pellets, placed in stainless steel planchets, was offered to the females. Upon removal of any remaining pellets, the containers were all examined for any spilled or moved pellets. If these were present, they were also removed and combined with the other urate pellets before final weights were recorded.

Uric acid in the fecal pellets produced during the third and fourth egg case cycles was determined with a modified uricase assay (Dubbs et. al, 1956; Cochran 1973). Correlations between diet, food consumption, days in the oothecal cycle and urate pellet consumption were determined using Spearman's nonparametric test for rank correlation.
Results

Adult female *Parcoblatta fulvescens* that had a good source of carbohydrate and limited nitrogen resources consume unmodified urate pellets. The relationship between diet and urate pellet consumption is given in Fig. 6.1. As dietary carbohydrate levels increase and protein levels decrease, the amount of urate pellets consumed increases. The increase in urate pellet consumption as protein levels increase by females on cellulose diluted diets may be due to increasing lipid concentration, since the dog food used for the protein source contains 8.0% fat. One female on the 34.4% protein diet and two on the 25% protein diet consumed less than 5.0 mg of the urate pellets. The consumption of urates by these females may have been partly due to nitrogen stress before the onset of the experiment. However, it is clear that as protein level of the standard diets is increased, urate pellet consumption ceases.

The females that consumed urate pellets voided only trace amounts of uric acid. The urate detected by the uricase assay was probably present in the feces due to incomplete absorption. All females on the 42% protein diet produced urate pellets, while only 2 of five females maintained on 34.4% protein excreted urates. One female on the 34.4% protein diet and three on the 25% protein diet consumed urate pellets.
Fig. 6.1. Relationship between dietary protein and urate pellet consumption. - Protein diets; — dextrose diets; — cellulose diets.
Results were not obtained from females maintained on the 5% protein, 86% cellulose diet due to mortality. Of 15 females placed on this diet, only one completed the third egg-case in the experiment. The others either failed to produce any egg-cases or died after the deposition of the second egg case of the experiment. Those females that did not produce egg-cases lived longer than those that apparently used up their metabolic reserves producing a single egg-case. This information suggests that females only store enough of a metabolic reserve to produce one egg case and that to form a successive egg-case an adequate food source must be available.

Females consuming urate pellets are shown in Fig. 6.2 a,b. One very interesting aspect of urate pellet consumption is that females that have been stressed for nitrogen consume urate pellets while carrying a newly formed egg case (Fig 6.2 b). In one case, a female that had been maintained on a 15% protein (50% Haydak dextrose, 50% dog food) diet for at least three months after the imaginal molt, and producing egg-cases was offered five unmodified urate pellets. This female had been in her harborage when the urate pellets were placed in the chamber. She located and began feeding on the pellets within five minutes. Within the following fifteen minutes all urate pellets were
Fig 6.2 Nitrogen stressed female Parcoblatta fulvescens with (a) and without egg cases (b) consuming formed urate pellets.
consumed. This rapid response to a nitrogen source was not expected. It suggests that females are able to perceive a nitrogenous resource.

Discussion

The data presented here show that reproductive female Parcoblatta fulvescens consume unmodified urate pellets when on a low nitrogen-high carbohydrate diet. Although it had been suggested earlier that this species may consume urate pellets as a nitrogen source (Cochran, 1979, 1982), attempts to feed unmodified pellets by themselves to starving individuals failed (Cochran, personal communication). It is interesting to note that females on the 10% protein-cellulose diet also consumed few pellets (Fig. 6.2). This diet may be close to a starvation situation since there is little protein or carbohydrate in this diet.

The presence of a utilizable carbohydrate source and low dietary nitrogen are essential for urate pellet consumption. Upon analysis, this situation is quite logical. When an individual is being starved, it is likely that it will mobilize its carbohydrate and lipid reserves for energy. After these stores have been depleted, it may begin to deaminate proteins to use for energy. The freed amino group can be incorporated into uric acid which may be either stored in the fat body or excreted. When Periplaneta
americana males are held on a 5% casein protein-cellulose diet, the fat body becomes overwhelmed with uric acid crystals (Cochran et al., 1979). If a similar situation occurs in Parcoblatta, a dietary source of uric acid in the absence of dietary carbohydrate would not provide utilizable nutrients. This would explain the failure to feed on urate pellets when they are offered as the only food source.

If carbohydrate and protein sources are freely available, female Parcoblatta fulvescens consume enough of each resource to meet their dietary needs and do not excrete uric acid (Chapter 5). If more protein than carbohydrates becomes available, the nitrogen intake may exceed the needs of the individual, resulting in urate excretion (Cochran, 1982).

In Periplaneta americana, fat body urates become depleted when the insects are held on a low nitrogen diet containing utilizable carbohydrate (Mullins and Cochran, 1975). This may also happen in Parcoblatta fulvescens when dietary nitrogen is limited. As fat body urates become depleted, individuals may begin to preferentially scavenge for high nitrogen foods, including urate pellets. This ability to selectively scavenge for and feed on high nitrogen food sources is suggested by the rapid response of nitrogen stressed, carbohydrate loaded individuals to a nitrogenous food source. Individuals are somehow able to
rapidly locate a high nitrogen food source when nitrogen has been restricted. Perception of nitrogenous compounds is probably olfactory since the individuals that were observed were in their harborages, and not within visual range of the nitrogen sources.

Since females feed on urate pellets, it is likely that individuals in a colony may also use these pellets as a food source in nature. This would represent an efficient means to share a nitrogen resource with all individuals within a colony. If some individuals have had access to high nitrogen foods, they may exceed the dietary nitrogen levels for urate excretion. Urate pellets from these individuals could be utilized by others that did not have access to nitrogen resources, but did have access to carbohydrates. These ideas are speculative and require the study of a naturally occurring colony for proof that urate pellets are used to transfer nitrogen between individuals.

If this strategy for transferring urates occurs naturally, it will represent a second means for cockroach species to transfer urates between individuals. The first strategy is paternal investment. *Xestoblatta hamata* females feed on urate secretions from the uricose glands of the males in a post-copulatory behavior (Schal and Bell, 1982). *Blattella germanica* males cement their spermatophore in place with a slurry of urates which are consumed by the female (Mullins and Keil, 1980). In both these species,
some of the urates are transferred to the oocytes. This means of urate transfer is limited with respect to the number of individuals that can share in the nitrogen resource. In the Parcoblatta pattern, individuals of all stages may be able to share urate nitrogen. This may ensure the development of not only the females and their developing offspring, but also all members of the present and future generations.
Chapter 7

Uric Acid in the Malpighian Tubules of Cockroaches

Abstract

Uric acid has been positively identified in the Malpighian tubules of three of the cockroach species known to void urates to the exterior: Parcoblatta fulvescens, Shawella couloniana and Symphloce hospes. The identification was made using enzymatic and histochemical techniques. During urate transport, uric acid will occupy the lumen of the middle and proximal regions of the Malpighian tubules, and will occasionally fill the distal tip. These urates are present as small spherules which form an opaque white slurry. This slurry is discharged into the hindgut in sufficient quantities to obscure the presence of food residues. Additional birefringent material within the tubule cell nuclei is present, the nature of which has not been determined, that may impinge on the urate transport mechanism.
Introduction

The woods cockroach *Parcoblatta fulvescens* (S. & Z.) has a unique method for voiding urates. These insects produce two types of fecal pellets. One is a normal dark brown pellet that presumably contains the residues of ingested foods; the other is a formed white pellet that contains a high concentration of urates. Only a few other species of cockroaches, all belonging to the subfamilies Blattellinae and Plecopterinae, have been shown to produce these urate pellets (Cochran 1973, 1976, 1979, 1981).

Uric acid has not previously been found in the Malpighian tubules of cockroaches (Nation and Patton, 1961; Wigglesworth, 1972). The tubules of other insects, however, are apparently the primary urate-secreting tissue (Wigglesworth, 1931 a,b,c; O'Donnell, Maddrell and Gardiner, 1983). This study is part of a series of investigations on the physiology of uric acid excretion in the wood cockroach, *P. fulvescens*. It's purpose is to present information on the morphology and histology of the hindgut of *P. fulvescens* with special regard to uric acid within the excretory system, including the Malpighian tubules.
Methods and Materials

Rearing of Parcoblatta fulvescens

Individuals from the VPI & SU cultures of Parcoblatta fulvescens, Shawella couloniana and Symproce hospes were used for all experiments. These cultures are currently being reared on dog chow and water ad. libitum. The insects used in these experiments were adult males. As many individuals as necessary to adequately demonstrate an event were used. These experiments were designed to describe the sequence of events that occur during the urate transport process with particular emphasis on the site of transport. Although all three species were used, this study concentrated on the Malpighian tubules of Parcoblatta fulvescens.

Examination of Hindgut for the Presence of Urates

Adult males were dissected in cold fat-body saline (Kurtti and Brooks, 1976). An insect pin was placed through the prothorax to immobilize the insect. A second pin, piercing the tip of the abdomen, allowed the insect to be stretched posteriorly. A sharp needle was used to tear the intersegmental membrane between the sixth and seventh abdominal segments. By pulling the tip of the abdomen
posteriorly, the hindgut was exposed. This procedure produced a preparation with little fat body tissue attached. Any remaining fat body and tracheae were teased from the hindgut with fine forceps. To remove the Malpighian tubules, the ampullae were grasped with fine forceps and torn from the hindgut.

Uric Acid Determinations

For all uric acid determinations, a standard uricase assay modified from Dubbs et al. (1956) was used. Uric acid was extracted from tissue homogenates in 0.5 ml of 0.6% Li$_2$CO$_3$ solution. The sample was stirred for 30 minutes with the temperature held constant at 60°C. The sample was centrifuged at 5,000 rpm in a Sorvall refrigerated centrifuge for 10 minutes and the supernatant decanted. The amount of uric acid in the supernatant was quantified, against a standard curve, by measuring the extinction of the characteristic absorption of uric acid at 292nm due to the degradation of urate by the enzyme uricase.

Preparation and Sectioning of Tissue

Hindguts with Malpighian tubules attached were dissected out of the insects in cold physiological saline. They were placed immediately in Carnoy fixative: 6:3:1
ethanol:chloroform:glacial acetic acid (Bancroft, 1975; Emmel and Cowdry, 1964). This fixative was used to prevent solubilization of the urates from the tissue. A dehydration series of 70, 80, 95% and two changes of absolute alcohol was used. The hindgut remained in each change step of the dehydration series for 25 to 30 min. The tissue was cleared in two changes of xylene and placed in a 50:50 xylene paraffin solution for 12 to 24 hours in a paraffin oven set at 65°C. Two changes of paraffin were used in the following 24 hours to ensure complete infiltration. The tissue was embedded in paraffin and allowed to harden for at least 24 hours before sectioning. 7um serial sections were cut on a standard microtome. The sections were floated onto albuminized slides with 70% alcohol and the slides placed on a slide warmer set at 45°C to dry.

Staining of Fixed Sections and Urate Identification

A variety of staining procedures were used to demonstrate different cytological features and urates. The major stain used was 1.0% eosin in 95% ethanol. This allowed for the staining of some cytological features without dissolving any urate crystals, which were normally detected using plane polarized light, that were present in
the tissue. To demonstrate nuclei, 0.1% methyl green in distilled water was used. This stain is specific for duplex DNA and did not stain urates.

To remove urate crystals, the slides were treated with 0.6% Li$_2$CO$_3$ for 30 minutes. Control slides were treated with 10% acetic acid in water, which prevented the solubilization of urates. Urate was detected by birefringence and by a urate specific staining procedure, Gomori's methenamine silver method (Gomori, 1952). Tissues lacking the white slurry in the hindgut and Malpighian tubules, indicating a non-urate excretory stage were used for controls.

Examination of Whole Tissue

In addition to sectioned tissues, whole mount preparations of the Malpighian tubules were prepared. Fresh tissue was examined in saline with phase-contrast microscopy. Whole mounts were fixed and stained for stellate cells (Wall, Oschman and Schmidt, 1975). Briefly, this involved placement of freshly dissected Malpighian tubules on an albuminized slide and fixing for 15 min. in Carnoy's fluid. After fixation, the slide was washed in distilled water and stained for 20 min. in 0.01% toluidine blue. A dehydration series of 70, 80, 95% and two steps of absolute alcohol for 5 minutes each was used. The
preparation was cleared in one change of 50:50 alcohol:xylene followed by two changes of xylene. The slide cover was mounted with Permount.

Time Lapse Photography

A group of females, on the 42% diet, were monitored with time lapse photography to determine when urate pellets were voided. Exposures were taken every 30 minutes on the second through fourth day after egg case drop. The times that urate pellets were voided were recorded from negative slides developed from the film. This helped to determine whether any daily pattern in the voiding process exists and if the pellets are produced in any specific sequence.

Results

Morphology of the Hindgut of Parcoblatta fulvescens

The general design of the hindgut of P. fulvescens is described in Fig. 7.1. The basic structure appears similar to that for Periplaneta americana (Wall and Oschman, 1970); Wall, et al., 1975; Bignell, 1981; Mullins, 1981) and Blattella germanica (Personal observation). Two major differences were seen. First, only two ampullae instead of
are present, as in *Blattella germanica* (Meyran, 1982). Second, the colon is enlarged and asymmetrically shaped (Fig. 7.1).

The ampullae connect to the alimentary canal at the junction between the midgut and ileum. The apical surface of the epithelia at this location in the midgut is fairly smooth and regular. At the pylorus, there is a dramatic transition along the apical membrane to long microvilli.

Examination of the Malpighian tubules reveals three distinct regions: distal, middle, and proximal (Fig 7.2, 7.3). It is possible that the middle segment may be further divided into lower and upper middle regions as in *Periplaneta americana* (Wall et al., 1975), but Meyran (1982) only observed three regions in *Blattella germanica*. The number of Malpighian tubules in *Parcoblatta fulvescens* was not counted but is quite large (probably greater than 100).

### Urate Transport Tissue

The presence of uric acid within the Malpighian tubule-rectum complex of *Parcoblatta fulvescens* was first noted in adult males. About one in fifteen individuals taken from the colonies showed a white slurry of material within the colon and rectum. Further dissections revealed that one in twenty males had the same white material present within the Malpighian tubules. Malpighian tubules from these
Fig. 7.2 Proximal (P) and middle (M) regions of the Malpighian tubules of *Parcoblatta fulvescens*. Unstained tissue under phase contrast, x200.
individuals were carefully checked to remove all fat body and analyzed for uric acid. Malpighian tubules lacking this white slurry were also analyzed. Uricase analysis revealed that this material was uric acid. An average of 3.8 ± 0.7 ug (mean ± S.D., N=5) of uric acid was present in the tubules removed from one adult male. No urates were detected in tubules from males that did not contain the white slurry.

Fresh Malpighian tubule tissue in saline was examined under a phase microscope. The white slurry within the Malpighian tubules was shown to consist of birefringent spherules. These spherules occupied the lumen of the entire length of the proximal and middle regions of the tubule and were occasionally found within the distal tip (Fig. 7.3 a,b). In fresh tissue, the urate spherules could be seen streaming towards the proximal segment as the tubules contracted and, if the hindgut remained attached, into the ampullae.

Histological examination of the hindgut has concentrated on the Malpighian tubules. The white spherules present in the tubule lumen react positively with methenamine-silver stain (Fig 7.4), again indicating the presence of urates. There appeared to be some artifacts created with this stain. Either the stain or the urates were apparently able to diffuse into the tissue itself. It may also be possible that urates within the Malpighian
Fig. 7.3 Distal region of the Malpighian tubule of Parcoblatta fulvescens containing urate spherules. Fresh unstained tissue. a, phase contrast; b, plane polarized light showing birefringence of spherules. x250.
Fig 7.4 Methenamine staining of sectioned Malpighian tubules of Parcoblatta fulvescens. Note dark staining area in lumen (L) and mild staining in nucleus (N). x250.
tubule cells were being stained, but the same diffusion of the reduced silver was also noted to be very strong in colon and rectal epithelia that were in contact with high concentrations of urates, and absent in adjacent cells that lacked this proximity. However, in some Malpighian tubule sections where the lumen did not contain spherules, a slight staining of the nuclei occurred (Fig 7.4). Whether this was urates or some other argentaffin material is uncertain.

Treatment of additional sections, stained only with alcoholic eosin, with lithium carbonate removed the birefringent crystals from the lumen of the tubules and the hindgut. Additional birefringent crystals remained within the cells of the Malpighian tubules (Fig 7.5 a,b), but these are assumed to consist of calcium or magnesium phosphates or carbonates that were observed by Wigglesworth and Salpeter (1962).

It can be concluded from the above results that uric acid is present in the Malpighian tubules in *Parcoblatta fulvescens* and that the tubules are the most likely site for secretion to occur. Examination of sections prepared from *Shawella couloniana* and *Sympleoce hospes* hindguts also revealed the presence of urates in the Malpighian tubules when the white spherules are present. This suggests that the mechanism for urate excretion is the same in these species.
Fig 7.5 Malpighian tubule section showing birefringent crystals within the tubule cells. Note cross-hatching of intracellular crystals (C) compared to urate spherules (S) in the tubule lumen. Methyl-green, eosin stain. a, phase contrast; b, plane polarized light. x250.
In one slide from Parcoblatta fulvescens, an unusual phenomenon was demonstrated (Fig 7.6 a,b). This slide was stained only with alcoholic eosin, and birefringent crystals, morphologically different from those just described, were present in the cells of the tubules. The crystals were apparently contained in large vesicles approximately the same size as the nuclei seen in the Malpighian tubule cells of other individuals (see also fig 7.5 a,b).

Other sections from this individual were stained with methenamine, so it was impossible to confirm the nature of this material. It is possible that the crystals were uric acid, and if so, the mechanism of urate excretion may involve vesicular transport. Since the nuclei could not be distinguished from these vesicles in this preparation, it is conceivable that these structures were the same. Additional examination of whole preparations fixed and stained with toluidine blue demonstrated birefringence within the nuclei that was similar to that within the vesicles. This preparation left some doubt that the intra-nuclear crystals were uric acid since the aqueous staining solution removed urate spherules from the tubule lumen. The exact nature of the birefringent material within the nuclei remains to be determined.
Fig. 7.6 Birefringent crystals contained in large vesicles (V) within the cells of the Malpighian tubules of Parcoblatta fulvescens compared to the urate spherules within the tubule lumen (S). Alcoholic eosin staining only. a, Phase contrast; b, Plane polarized light. x250.
In one aging adult male, differently structured crystals were found within the lumen of the Malpighian tubules (Fig. 7.7 a,b). It was not adequately demonstrated that these crystals were urates, but the shapes are characteristic of sodium or potassium urate crystals found in gout. The tubule that contained the crystals had degenerated. It is possible that normal urate spherules were formed within the tubule, but since the tubule was occluded the crystals remained and transformed from spheres to rod-shaped crystals.

Hindgut Contents

The contents of the hindgut were examined in both transporting and non-transporting insects. The rectal contents are shown in Fig. 7.8 a,b,c,d. Rectal contents from non-transporting individuals show both amorphous and cellular materials. Some of the cellular matter may be birefringent (Fig 7.8 b). The rectal lumen containing a forming urate pellet demonstrates that the majority of the contents are large spherules of urate. These spherules are normally larger than the spherules in the Malpighian tubules, but may also be the same size. Among the urate spherules, material similar to the rectal contents of a non-transporting individual may be seen.
Fig. 7.7 Birefringent crystals contained within the lumen of the Malpighian tubules of an older adult male Parcoblatta fulvescens. a, Phase contrast; b, Plane polarized light. x250.
Fig. 7.8 a, b. Rectal contents from an individual not excreting urates under phase contrast (a) and plane polarized light (b). x100.
Fig. 7.8 c, d. Rectal contents from a urate excreting individual under phase contrast (c) and plane polarized light (d). x200.
To further investigate the nature of the rectal contents and the urate containing fecal pellets, whole, freshly collected, fecal pellets were placed in 0.6% Li$_2$CO$_3$. The fecal pellets remained in the solution for two hours. The urate and non-urate pellets looked superficially different at the onset of this procedure (Fig 7.9 a,b). As uric acid was extracted, the residue contained in the urate pellet appeared to consist of the same material as the normal fecal pellet (Fig 7.9 c,d). This indicates that the normal flow of digested material through the alimentary canal does not cease for urate pellet production.

It is possible to begin to describe the path of the urates within the hindgut lumen from the slides that have been prepared. In one case, it was possible to obtain a hindgut with the majority of urates within the Malpighian tubule lumen. Traces of urate crystals can be observed within the rugae of the colon and between the rectal pads of almost all hindguts examined, including those that did not contain forming pellets. Other hindgut sections revealed a flow of the urate slurry from the tubules through the ileum and colon to the rectum. This is apparently a continual process since none of the sections demonstrated the presence of urates in the tubules and rectum and not the colon.

Analysis of the data from the time-lapse photography does not indicate that the voiding of uric acid occurs at any specific time of the day. This suggests that the
Fig. 7.9 a, b. Freshly collected urate (left) and non-urate (right) pellets, before (a) and immediately after (b) addition of 0.6% Li₂CO₃ solution. x40.
Fig. 7.9 c,d. Urate (left) and non-urate (right) pellets, one (a) and two (b) hours following addition of 0.6% Li$_2$CO$_3$ solution to extract uric acid. x40.
regulation of transport relies solely on internal factors. The urate pellets and normal pellets were not voided in a random pattern, nor did they alternate. Occasionally a urate pellet would be voided between two groups of normal pellets. In two instances, the only pellets voided on the third day after egg case drop were urate pellets. The average time between the voiding of any two pellets was approximately three hours. These data gave some information on the voiding of the pellets, but very little on the functioning of the Malpighian tubules.

Discussion

The results presented in this chapter demonstrate that uric acid occurs in the Malpighian tubules of three species of cockroaches, *Parcoblatta fulvescens*, *Shawella couloniana* and *Simiope hospes*. Urates are not, however, always present in the Malpighian tubules of these species. This indicates that there is a form of regulation to the urate excretory process. The presence of urates within the Malpighian tubule in these species was not expected, partly due to the lack of urates in the tubules of other cockroach species (Nation and Patton, 1961; Srivastava and Gupta, 1961).
However, uric acid is the major nitrogenous excretory product of most insects (Wigglesworth, 1972). In all urate excreting species in which the Malpighian tubules have been tested for the presence of uric acid, uric acid has been demonstrated within the tubule lumen (Wigglesworth 1931a, 1972; Buckner, Caldwell and Reinecke, 1980; O’Donnell et al., 1983). It is normally present in the proximal third of the Malpighian tubule as a white slurry (Wigglesworth, 1972). The presence of urates within the tubules of *Parcoblatta fulvescens*, *Shawella couloniana* and *Simloce hospes* is strikingly different. The urate slurry occupies almost the entire length of the Malpighian tubules, and essentially all tubules are involved.

Uric acid may be passively transported across the distal segment of the Malpighian tubules in *Rhodnius* (Maddrell and Gardiner, 1974). However, the mechanism of transport of urates in *Rhodnius prolixus*, *Pieris brassicae*, *Schistocerca gregaria*, *Glossina morsitans* and *Tenordora sinesis* is apparently an active process, and occurs in the proximal third of the tubules. It is highly probable that the transport of urates in urate-voiding cockroach species is also an active process, but the presence of urate spherules within the entire length of the tubules suggests that the middle region is largely responsible for urate transport. The proximal region of the tubule may also be involved in the transport of urates, but the presence of
urate spherules in the distal tip of the tubules is most likely due to a 'back-flow' of urates into the tip as the tubules contract.

The urate spherules pass into the ampullae where they are discharged into the hindgut. It is apparent that the urates are admixed with the food residues from the midgut. As this mixture is passed into the colon, the urate spherules frequently become larger. The reason for the enlarged colon (approximately twice the diameter of the colon found in *Blattella germanica*) in the urate transporting cockroach species (Present paper and Cochran, personal communication), is unknown. Since it is apparently enlarged only in urate excretors, this design probably plays a major role in the urate excretory process. Further study of the colon of these species may elucidate the reason for the enlargement.

It is possible that other materials may be transported with uric acid by the Malpighian tubules during the urate excretory phase. If nutritional materials are present in the Malpighian tubule during urate secretion, which are not reabsorbed in the hindgut, this would be quite significant since urate pellets are consumed by nitrogen deficient females (Chapter 6).
The fact that uric acid is not always present within the Malpighian tubules lends support to the hypothesis that urate excretion plays a regulatory role in the nitrogen economy of cockroaches (Cochran, 1979, 1981, 1985). During the urate excretory phase, a large amount of urate spherules accumulate in the Malpighian tubules and hindgut. These urates are secreted in quantities great enough to obscure the presence of food residues within the hindgut and in the final fecal pellet produced. This indicates that the excretory process occurs quite rapidly so that invariably either urate-containing or normal fecal pellets are produced. This observation earlier led to the assumption that the formation of the urate pellets involved the closing of the pyloric valve to keep the urates from admixing with the food residues (Cochran, 1973).

It is quite probable that the process of urate excretion is similar in all cockroach species that produce discrete urate pellets. However, it may be interesting to examine this process in one of those species that excretes urates admixed with the fecal material; such as Ischnoptera deropeltiforms (Cochran, 1979), I. rufa debilis or I. rufa rufa (Cochran, 1981). These species produce urates in the excreta at a rate of approximately 2.0% by weight. It is possible that urate excretion is a continual process in these species. It can be hypothesized that the Ischnoptera group may be similar to the Parcoblatta group in urate
excretion, but lack a critical step in the regulation of uric acid excretion. Further study of urate-excreting cockroaches is needed to define the mechanism(s) by which they regulate urate excretion.

The presence of the birefringent crystals within the nuclei deserves comment. The nature of these crystals has not been established, but the possibility that they may contain uric acid exists. If these are urates, they may be tightly bound to some material which prevents their solubilization in aqueous solutions. Their presence may indicate that the nuclei are involved in the mechanism by which urates are transported across the tubule wall. It must be mentioned, however, that other birefringent materials may be present in nuclei. Spindle fibers have been shown to be birefringent and can be measured by this property (Schaap and Forer, 1984). In addition, proteinaceous intranuclear crystals are present in the midgut epithelium of the whirligig beetle *Gyrinus marinus* (Gourantant and Thomas, 1974) and in the Cheleutopteran, *Carausius morosus* (Thomas and Gourantant, 1980). It is possible that any regular crystalline array may have birefringent properties. Further study is needed to determine the nature of the birefringent crystals within the Malpighian tubule cells of *Parcoblatta fulvescens*. 
Chapter 8

Discussion and Conclusions

Examination of the overall results from this study must be made to help clarify the role of uric acid excretion in the physiology of Parcoblatta fulvescens. This will be done primarily in the context of the nitrogen balance equation presented by Cochran (1985):

\[
NB = NI - (UN + FN + SN + EN)
\]

Where \(NB\) is the nitrogen balance, \(NI\) is nitrogen intake, \(UN\) is urinary nitrogen, \(FN\) is fecal nitrogen, \(SN\) is stored nitrogen and \(EN\) is egg nitrogen. The potential effects of dietary carbohydrates on nitrogen balance will also be considered. Finally, the urate excretory process will be summarized and the possible regulation of this process will be discussed.

The first aspect of this equation to be explored is the effect of nitrogen intake on nitrogen balance and urate excretion. As has been mentioned previously, a 'break-even point' with respect to urate excretion may be more correctly defined as the level of protein ingested which is just adequate to meet the needs of a specific developmental stage. When the level of dietary protein or nitrogen is
increased above the break even point, the insect is placed in a state of positive nitrogen balance. In *Parcoblatta fulvescens*, increases in protein intake increase the amount of uric acid excreted in an apparently linear fashion (Fig. 4.3). Thus, as nitrogen intake increases, the amount of urinary nitrogen in the form of urates also increases. When the amount of nitrogen ingested is below this threshold level, the individual is in a state of negative nitrogen balance and urate excretion will not occur.

The amount of urate nitrogen stored in the fat body of *Parcoblatta fulvescens* remains relatively constant as long as the insect is in a state of positive nitrogen balance or is ingesting a maintenance level of protein (Cochran, personal communication). When reproductive females are given a choice of protein, carbohydrate or cellulose diets, they are able to regulate their nitrogen intake so that UN, FN, and SN remain relatively constant. However, during the oothecal cycle the consumption of protein changes slightly on a day to day basis (Fig. 5.1). This indicates that the nitrogen demand may vary depending in the amount of proteins and urates being transferred to the developing ootheca. Thus, as more nitrogen is transferred to the egg case, both EN and NI increase. The other variables in the equation remain constant as long as adequate food sources exist.
When the diet is deficient in proteins, the nitrogen balance of reproducing females becomes negative. The nitrogen needed for the developing oocytes is taken from the urates stored in the fat body. If another nitrogen source becomes available, such as urate pellet nitrogen, reproductive females will consume sufficient amounts to meet their nitrogen demand, but this will not result in urate excretion. To put urate pellet consumption in context with the nitrogen balance equation:

\[ NB = \text{FoN} + \text{UPN} - (\text{UN} + \text{PN} + \text{SN} + \text{EN}) \]

Where FoN is food nitrogen and UPN is urate pellet nitrogen. This is similar to the distinction between FoN and PN (paternally-derived nitrogen) in the NI portion of the nitrogen balance equation (Cochran, 1985).

One major distinction should be reiterated in the difference between urate pellet consumption and paternal investment of urates. With paternal investment, primarily reproducing females and their offspring receive the benefit of the nitrogen reserves in the urates transferred by the males. With *Blattela germanica*, if the reproducing females do not need these reserves, they may discard the spermatophore and urates where other individuals could consume these urates and derive some benefit from them (Mullins, personal communication). However, since urate transfer by *Xestoblatta hamata* is a post copulatory
behavior, only the female is able to derive benefit from the urates. In *Parcoblatta fulvescens*, all members of a colony, except early instars which do not excrete significant amounts of urate (Cochran, 1979), may be capable of producing urate pellets, and all individuals may have equal opportunity to consume the urate pellets as a nitrogen resource.

To demonstrate consumption of urate pellets, it was necessary to provide a diet containing significant amounts of utilizable carbohydrates. This raised a very important question: What is the effect of metabolizable dietary carbohydrate on the nitrogen balance of *Parcoblatta fulvescens*? This effect is suggested in Fig. 6.1 on urate pellet consumption and has been addressed in Chapter 6, but will be explored further here in terms of the nitrogen balance and economy of *Parcoblatta fulvescens*. Due to the preliminary nature of these findings, the discussion below remains partially hypothetical and requires further study.

Under conditions of nitrogen stress, when there are insufficient dietary carbohydrates to meet the energy demands of an individual, carbohydrate stores will be utilized for energy. When these stores have been depleted, the individual will begin to metabolize proteins for energy. The nitrogen from deamination of proteins will be converted to uric acid for storage or excretion. Thus, even though
nitrogen intake is restricted, the amount of nitrogen available exceeds the metabolic demand. To show this event in the nitrogen balance equation, metabolic nitrogen pool (MN) and nitrogen storage in the form of urates (SU) are equated to SN:

\[
NB = NI - (UN + PN + (MN + SU) + EN)
\]

When dietary nitrogen and carbohydrates are restricted, MN will decrease due to the demand for carbon and SU will increase. In *Parcoblatta fulvescens*, SU may have a maximum value and when this limit is reached, urate pellet excretion will occur, increasing UN.

In contrast, when there are sufficient carbohydrates in the diet, the individual may use these carbohydrates for energy. If nitrogen intake is restricted, the nitrogen stored in fat body urates may be utilized for metabolic nitrogen. The net value of the metabolic nitrogen pool, however, may not change. Urate pellet excretion will not occur, thus reducing UN to a minimum.

The possible effect of carbohydrates in diets containing higher protein levels on the nitrogen balance of *Parcoblatta fulvescens* is less apparent. The diets that have been used to determine the effect of increasing protein levels on urate excretion (Cochran 1981; Cochran and Mullins, 1982; and present study, Chapter 4) did not test the effect of the decreasing carbohydrate and lipid levels
as protein concentration increased. It is possible that the increase in protein consumption with increasing protein level in the diet may be, in part, due to the insects need for energy containing compounds.

To obtain sufficient carbon for energy, the insect is required to consume more nitrogen. This puts the insect in a positive nitrogen balance. As more protein is consumed, it is deaminated to recover the carbon chain for energy production. The excess nitrogen is incorporated into uric acid for storage and excretion. Thus, in the nitrogen balance equation, NI is increased due to a lack of carbohydrate increasing SN and UN. Some increase in FN may occur due to incomplete absorption of proteins. In addition, an increase in protein and urate transfer to developing oocytes (EN) by reproductive females may be possible.

If the level of dietary carbohydrate meets or exceeds the demand, wood cockroaches may be able to regulate the nitrogen intake by controlling total food consumption. This became apparent in the diet preference study. These *Parcoblatta fulvescens* females were capable of regulating not only the amount of protein, but also carbohydrate consumed. In addition, they did not excrete uric acid under these conditions. In terms of nitrogen balance, they were neither nitrogen loaded nor stressed, but maintained their nitrogen intake in equilibrium with nitrogen demand.
In summary, the wood cockroach, Parcoblatta fulvescens may have two means with which to regulate their nitrogen economy. If sufficient carbohydrates are available they can control their nitrogen intake. When there is excess nitrogen available, either dietary or metabolic, they can excrete uric acid.

When it is necessary to excrete urates, enough uric acid is mobilized from the fat body to produce at least one formed urate pellet. These urates are transported in the hemolymph to the Malpighian tubules. They appear to be carried in solution, or possibly bound to protein, since birefringent crystals have not been seen in freshly collected hemolymph (Lembke, personal observation). The transport of the urates across the tubule wall may also be in soluble form, unless the birefringent crystals within the nuclei are urates. Whether urate transport is an active process remains to be determined.

The urates within the tubule lumen form small white spherules that may be present within the distal tip of the Malpighian tubule. They are moved into the ampullae by contractile movements of the tubules. This may be facilitated by the flow of the primary urine. Once the spherules reach the ampullae, they are discharged into the hindgut in sufficient quantities to obscure the presence of food residues passing into the ileum from the midgut. Some
modification of the spherules in this admixture may occur in the colon. Frequently, the spherules grow larger, attaining or exceeding the size of those spherules present in the fat body. From the colon, the admixture is passed into the rectum where it is apparent that some water resorption occurs to compact the urates into the final pellet which characteristically contains indentations from the rectal pads.

The process of urate pellet excretion appears to be tightly regulated. This is apparent from the fact that the Malpighian tubules do not always contain uric acid spherules and in the rapidity of pellet formation following urate secretion into the tubules. The possible regulation of urate transport has been discussed in Chapter 4. Additional suggestions on the initiation of the transport process will be considered here. As mentioned above, urate excretion is a response to positive nitrogen balance. Before urate excretion can occur, there must be sufficient uric acid stored within the fat body to produce one or more urate pellets. This indicates that the fat body maintains urate concentration within a specific range.

The insect will attempt to store a minimum level of uric acid in the fat body. Below this level, more nitrogen, if available, will be consumed to increase the amount of stored urates. If more protein or nitrogen is consumed, the insect will continue to synthesize and store urates until
enough are available to be transported to the Malpighian tubules. Regulation of the transport process may begin with hormonal or neural signals from the fat body that indicate whether storage or mobilization for excretion are needed.

Additional study of the physiology and ecology of Parcoblatta fulvescens is needed to further define the unusual urate handling mechanism of this and other urate pellet-voiding species. Examination of the influence of carbohydrates on the nitrogen balance of cockroaches may be quite useful. Several questions still remain to be answered: What other nitrogenous materials do wood cockroaches excrete? What is the mechanism(s) by which urate pellet-voiding species regulate urate storage and excretion? and finally, Does urate pellet excretion occur in nature and what is its impact on the nitrogen economy of native colonies?
Literature Cited


Wigglesworth, V. B. 1931b. The physiology of excretion in a blood-sucking insect, Rhodnius prolixus (Hemiptera, Reduviidae).--II. Anatomy and histology of the excretory system. J. Exp. Biol. 8: 428-442.


## Appendix A

### Wayne Dog Food Composition

(Allied Mills, Inc., Chicago, IL 60606)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, %</td>
<td>25.0</td>
</tr>
<tr>
<td>Fat, %</td>
<td>8.0</td>
</tr>
<tr>
<td>Linoleic Acid, %</td>
<td>2.0</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>2.1</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>1.4</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.18</td>
</tr>
<tr>
<td>Iron, mg/kg</td>
<td>220.0</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
<td>13.0</td>
</tr>
<tr>
<td>Manganese, mg/kg</td>
<td>80.0</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>140.0</td>
</tr>
<tr>
<td>Iodine, mg/kg</td>
<td>1.4</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin A, IU/kg</td>
<td>11500.0</td>
</tr>
<tr>
<td>Vitamin D, IU/kg</td>
<td>3800.0</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
<td>50.0</td>
</tr>
<tr>
<td>Vitamin B12, mg/kg</td>
<td>0.02</td>
</tr>
<tr>
<td>Folic Acid, mg/kg</td>
<td>0.90</td>
</tr>
<tr>
<td>Thiamine, mg/kg</td>
<td>4.7</td>
</tr>
<tr>
<td>Riboflavin, mg/kg</td>
<td>4.1</td>
</tr>
<tr>
<td>Pyridoxine, mg/kg</td>
<td>7.0</td>
</tr>
<tr>
<td>Pantothenic Acid, mg/kg</td>
<td>11.5</td>
</tr>
<tr>
<td>Vitamin</td>
<td>Amount (mg/kg)</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Niacin</td>
<td>42.8</td>
</tr>
<tr>
<td>Choline</td>
<td>1300.0</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.13</td>
</tr>
</tbody>
</table>
The vita has been removed from the scanned document