Physiology of Environmental Adaptations and Resource Acquisition in Cockroaches

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Abstract

Cockroaches are a group of insects that evolved early in geological time. Because of their antiquity, they for the most part display generalized behavior and physiology and accordingly have frequently been used as model insects to examine physiological and biochemical mechanisms involved with water balance, nutrition, reproduction, genetics, and insecticide resistance. As a result, a considerable amount of information on these topics is available. However, there is much more to be learned by employing new protocols, microchemical analytical techniques, and molecular biology tools to explore many unanswered questions.

OVERVIEW

Mycetocytes: nonpathogenic microorganisms that are described as mycetocyte symbionts located in specialized cells referred to as mycetocyte cells

Cryoprotectants: substances that protect biological tissue by lowering body fluid freezing points (supercooling), thus avoiding ice crystal formation: sugar alcohols, polyols, antifreeze proteins

A wealth of information is available on cockroaches, primarily domestic pest species and others that can easily be maintained in laboratory or museum facilities. Much of that information can be found in texts by Cornwell (22), Guthrie & Tindall (46), Bell & Adiyodi (8), Rust et al. (110), and Bell et al. (9). The primary focus of these references is on the major pest species (primarily German and American cockroaches), except for Bell et al. (9), who have provided an excellent discussion on the diversity of cockroaches based on their ecology, behavior, and natural history (1,500 references). Cockroaches are best described as generalized insects with chewing mouthparts. Their life cycle is typical of other hemimetabolous insects, consisting of egg, nymph, and adult stages. Their reproductive strategies are quite variable. Their eggs are typically enclosed in oothecae, which some species deposit shortly after formation (oviparous). Others carry their oothecae externally until or near hatching (also oviparous), or internally (ovoviviparous). Finally, one species incubates and provisions developing embryos prior to their emergence as first instar nymphs (viviparous).

Available information on cockroaches clearly indicates that they are a diverse group of insects. Cockroach-like fossils have been found in late Carboniferous deposits (68), although they first appeared quite early in insect evolution (early to mid-Mesozoic) (9, 45, 132, 135). They have adopted many lifestyles, live in a variety of habitats, and feed on diverse food materials. In comparing well-known insect feeding groups, Dow (30) designated cockroaches stereotypic "generalists," placing them in a central position among all insect dietary types (solid/liquid and plant/animal substrates) based on their apparent capabilities as omnivores (**Figure 1**). It is increasingly apparent that a great deal of their success in nutrient procurement, digestion, and metabolism may be attributed to their symbiotic relationships with both gut microbes (24, 60, 61, 85, 117, 139) and mycetocyte bacteroids (19, 28, 64, 79, 100), which have enabled them to extend the scope of their nutritional and metabolic capabilities. In fact, the physiological and biochemical relationship between their mycetocytes and stored fat body urates appears to provide the cockroach host with a resource for mobilizing and utilizing these urate stores, which are generally considered a major metabolic waste product (45). These stored urates can also serve as a resource for both maternal and paternal nitrogen investment in their progeny (83). Literature on termite biology draws similarities between wood-feeding cockroaches and some termite species (9). These similarities have been substantiated given that morphological and molecular analyses indicate that termites are a sister group to the wood-feeding cockroach genus *Cryptocercus* (23, 26, 56, 138). Therefore, an examination of resource acquisition and environmental adaptations in cockroaches could also be informative for termite studies.

Although most cockroach species are considered tropical (found primarily in environments that are humid and warm year-round), some possess adaptations that enable them to thrive in hot and also very dry (desert) environments (9, 33). These include physiological and behavioral adaptations, water acquisition (capturing water vapor), and conservation measures (cuticular and respiratory). Other species are capable of surviving in extremely cold climates, utilizing a unique combination of sugars, sugar alcohols (cryoprotectants), and nucleating proteins (47, 142). Because of their generalized structure and the ease by which they can be maintained in laboratory cultures, certain cockroach species have been widely used for classroom instruction and experimentation. Students have dissected cockroaches, observed the beating dorsal vessel, measured nerve cord activity, and carefully dissected out other systems for observation or experimentation. Cockroaches contribute significantly to our understanding of basic insect physiology/biochemistry and behavior. Specializations that illustrate the functional diversity of these insects are seen in a cockroach species that jumps with the use of saltatorial legs (*Saltoblattella montistabularis*) (13, 102); a luminescent cockroach that exhibits defensive, Batesian, and interordinal mimicry (*Lucihormetica luckae*) (133, 134); and a specialized plant pollinator (*Clusia* aff. *sellowiana*) (131). Cockroaches

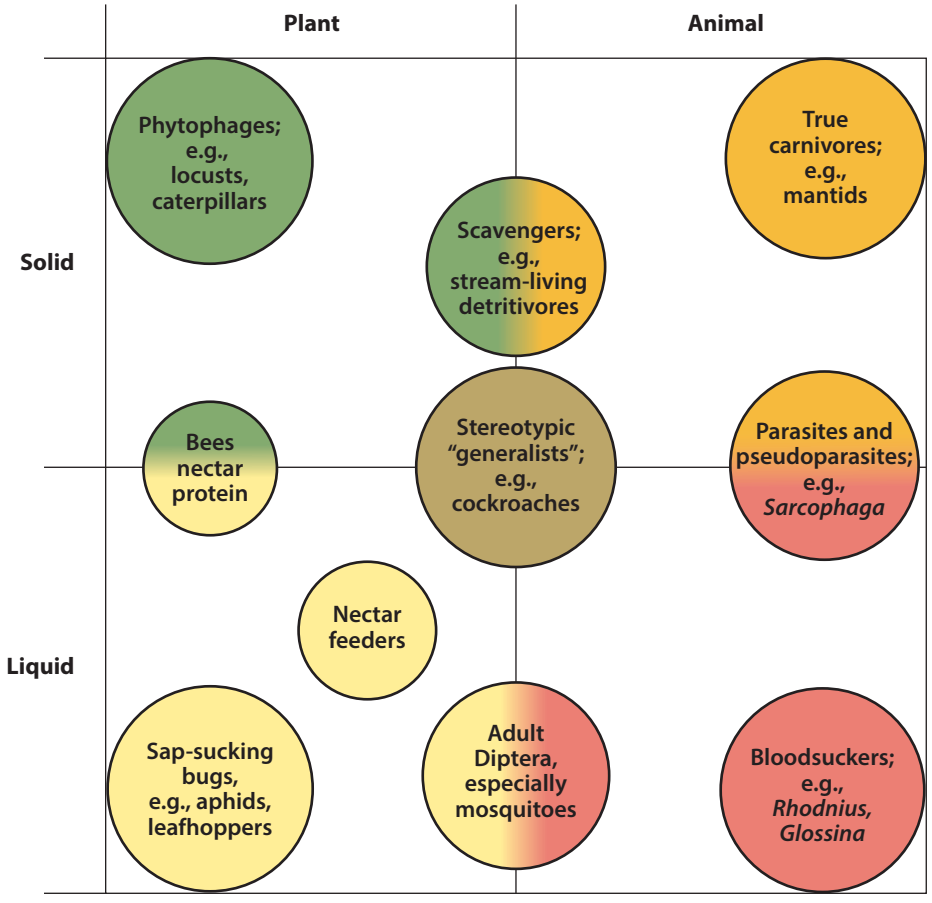

Figure 1

A proposed feeding classification system based on four classes of feeding types: solid/plant feeders, solid/animal feeders, liquid/animal feeders, and liquid/plant feeders. According to this classification scheme, cockroaches are located centrally, as stereotypic "generalists"/omnivores. Microbes can be included as direct food sources in a broad definition of omnivore (9). Adapted with permission from Reference 30.

are currently used as research models in evolutionary biology (52), in robotics (121), in materials science (140), in (bio)fuel cell technology (105), and in nanotechnology (150).

The intent of this review is to summarize the physiological and biochemical adaptations that have allowed these insects to assume a variety of lifestyles and inhabit a variety of habitats. (**Figure 2**). A useful perspective in examining the diverse adaptive changes allowing cockroaches to fill a variety of ecological niches is to consider these changes on the basis of their bioenergetics. Downer (31) has provided a thoughtful perspective on this topic, outlining the physiological and environmental factors that must be considered. To achieve a critical positive balance, all metabolic requirements for such processes as growth, mobility, and reproduction must result in a positive balance with all related energy losses. With a focus on nutrient procurement as a significant factor in insect bioenergetic relationships, the designation of cockroaches as generalists is fitting in exploring the diversity of the cockroach lineage. This adaptive flexibility has enabled them to utilize a variety of food substrates efficiently.

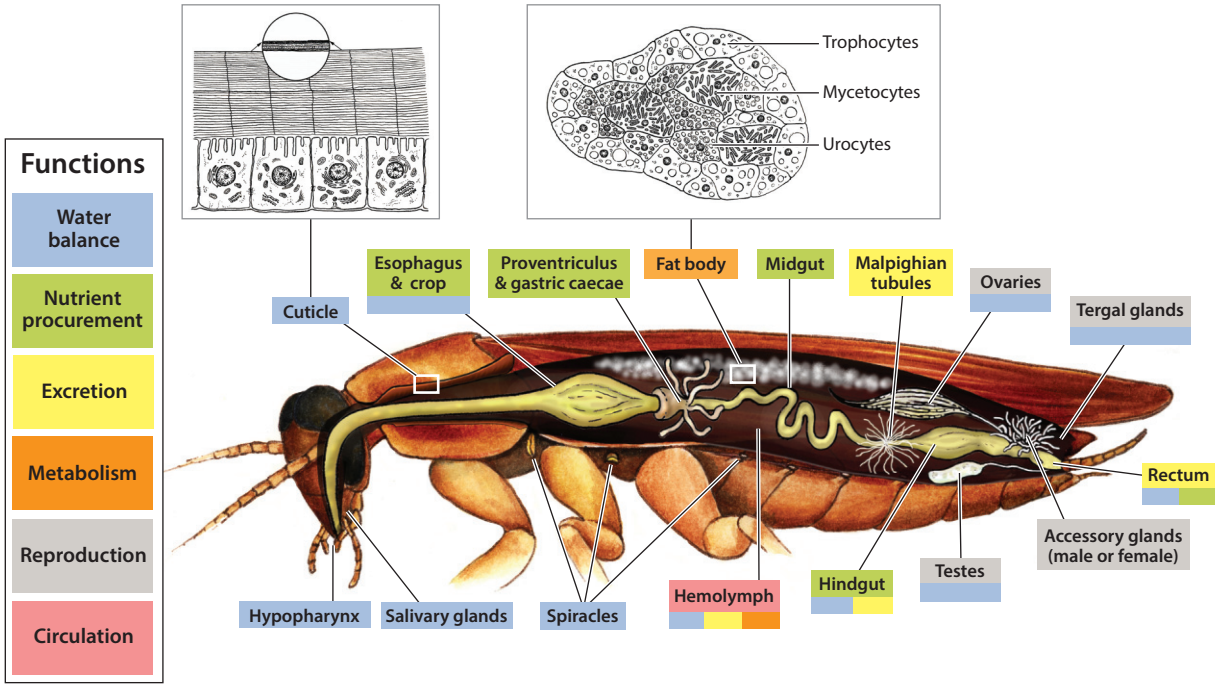

Figure 2

Physiological adaptations and their relationships in a generalized cockroach. Color coding indicates associated functions and their relationships with the cockroach systems. Water balance (*blue*) includes (*a*) uptake by ingestion (drinking and eating), water vapor (hypopharynx/*Arenivaga* spp.); (*b*) respiratory losses (cuticular and spiracular); (*c*) excretory losses (feces); and (*d*) secretory losses (accessory and tergal glands). Nutrient procurement (*green*) includes (*a*) food processing (physical, mouth parts and proventriculus; and biochemical, digestion and absorption in the midgut), (*b*) recycling (microbial symbiont contributions and absorption in the hindgut), and (*c*) excretion (water and solute recovery in the rectum and waste elimination). Excretion (*yellow*) includes the involvement of (*a*) the Malpighian tubules, where solutes and water are filtered from the hemolymph (primary urine), (*b*) the hindgut, where urine and midgut effluents are admixed with the gut symbionts (some absorption of solutes occurs), and (*c*) the rectum, where water and solute recovery and waste elimination occur. Metabolism (*orange*) includes (*a*) fat body (three cell types: trophocytes, general intermediary metabolism; urocytes, storage of urates participating as a nitrogen store and an ion sink; and mycetocytes, nitrogen recycling) and (*b*) hemocytes (storage and metabolism of intermediary metabolites). Reproduction (*gray*) includes (*a*) female ovaries (egg production) and female accessory glands (provide materials for egg case construction) and (*b*) male testes (sperm production), male accessory glands (form spermatophores and in some species may store urates that are shared with females at mating), and tergal glands (which may perform a variety of functions, providing pheromones, nuptial gifts, etc.). Circulation (*pink*) includes (*a*) hemolymph (aqueous medium providing transport of solute molecules), (*b*) excretion (in association with the Malpighian tubules, hindgut, and rectum), (*c*) water reservoir (in association with crop, salivary glands, etc.), and (*d*) cold hardiness (constituents that may convey adaptations to cold by lowering the body supercooling points or that may serve as ice-nucleating factors).

FACTORS INFLUENCING WATER BALANCE

The regulation of water balance and thermoregulation are primary requirements for maintenance of homeostasis, which may be costly (18). A driving force enabling organisms to maintain a relatively stable internal environment is the maintenance of water balance, achieved between fluid uptake (in food and drink) and loss (transpiration, excretion, and secretion) (**Figure 2**).

Water Acquisition

Water acquisition is primarily achieved either by drinking or through food intake and metabolism. Given that cockroaches are omnivores, dietary water obtained from food can be quite variable.

Once water is taken into the alimentary canal, it must be absorbed through physiological processes in the digestive and excretory systems (22, 33, 46). In addition to dietary uptake, a desert-dwelling cockroach (*Arenivaga investigata*), adapted to living under extremely xeric conditions, is able to absorb water vapor from unsaturated atmospheres above 82% relative humidity by using its hypopharyngeal bladder (95). Additional studies by O'Donnell (93) indicated that the condensation of water on these bladders is attributable to a specialized cuticle on these structures. Examination of the microionic environment of the proximal bladder cuticle indicated that there was not a significant amount of water associated with low-molecular-weight solutes (Na^+). O'Donnell (93) proposed an in vivo model involving recycled frontal body fluid that alters the bladder cuticle affinity, enabling the release of condensed water that can then be ingested. Currently, understanding of the absorption and delivery processes involved in this species is incomplete, but they are worthy of more study.

Water Loss

Water conservation presents insects with physiological problems when they are exposed to variations in relative environmental temperatures and relative humidities. Although a majority of the Blattaria are tropical or semitropical, there are species that have adapted to less humid environments. Water loss in insects has been of considerable interest for decades. Studies of water loss were pioneered by J. Arthur Ramsay and others (38), and a number of researchers have investigated it since that time. The physiological mechanisms that regulate water loss include cuticular, respiratory, and excretory processes. Most investigations have considered changes in water content, rates of water loss, and tolerance of water loss (desiccation resistance) (18). These studies present significant technical challenges because they must be conducted under controlled environmental conditions (temperature and relative humidity). The technical approaches to these studies have been quite varied, including employment of a variety of respirometers and radiotracer methodologies (^3H_2O and $^{14}CO_2$) (2, 91). Separating cuticular loss from respiratory loss, which may be affected by both behavioral and physiological factors, has been a major complicating factor in evaluating water loss (2, 43, 71).

The results of early work comparing cuticular water losses with increased temperature were observed and attributed to disruption of the nonpolar lipid epicuticular layer (3). However, Gibbs (39) provided an analysis on the thermodynamics of cuticular transpiration, examining the hypothesis that a temperature-transition phenomenon could be ascribed to temperature-dependent changes in epicuticular lipids responsible for increased cuticular water losses. As a result, he suggested that experimentation on models designed to examine changes in activation entropy for diffusion through the cuticular matrices under various experimental conditions could be useful (39). Some of the work that has led to developing a more comprehensive understanding of cuticular water loss include publications by Machin & Lampert (72), Appel & Tanley (3), and Noble-Nesbitt et al. (91). While examining the energetics of water diffusion through *Periplaneta americana* cuticle by measuring the effects of temperature on pronotal discs, Machin & Lampert (72) found that in vivo water permeability at 10–35°C was an order of magnitude lower than reported earlier. They concluded that the (lipid-soluble) cuticular barrier consisted of a novel heat-sensitive molecular structure rather than a conventional oriented monolayer. Appel & Tanley (3) compared body-water composition and water-loss rates for five *Blattella germanica* body color mutants and observed differences in their water permeability. They concluded that properties of the intact nonlipid layer may render the cuticle less permeable to water because of the degree of sclerotization (more tanned cuticle). In addition, they found hexane-extracted lipids from the dark mutants had lower cuticular permeabilities than those from the lighter mutants, indicating that the observed

differences in permeability were due to differences in the cuticle rather than the amount or composition of cuticular lipids (3). Measurements of water and carbon dioxide loss from *P. americana* by employing radiotracers (^3H_2O and $^{14}CO_2$), performed by Noble-Nesbitt et al. (91), indicated that although there was no obvious relationship between cuticle permeability and initial body mass after 72 h, there was significant lowering of cuticular permeability. They also noted a marked, sudden change in permeability when a humid airstream was changed to dry air. Because of their experimental setup, they were able to demonstrate that tracheal water loss was only a small amount of total water loss (averaging 3%). Clearly, there is much to be learned about cuticle composition and structure as they relate to the physiology of water conservation. Newer techniques, such as higher resolution and fluorescent microscopy and microchemical analytical procedures, should be helpful in this regard.

Insect respiratory physiology and water balance has been a topic of study for many years. Major technical advances have provided more sensitive and precise methods that have contributed to a thriving and vigorous area of research (104). A number of reports address cockroach respiration rates associated with the effects of temperature, relative humidity, metabolic rates, and oxygen/carbon dioxide levels (pO_2, pCO_2). Some insects exhibit discontinuous gas exchange cycles (DGCs). New technologies including highly sensitive means of measuring CO_2 and water loss have indicated that many insects may cycle their respiration. However, the adaptive benefit and evolutionary origin of this behavior remain unclear (66, 76). Currently, investigations are focused on three hypotheses to explain the physiological purpose of DGCs: (*a*) to limit respiratory water loss (hygric hypothesis), (*b*) to enhance gas exchange in subterranean environments (chthonic hypothesis), and (*c*) to limit oxidative damage (oxidative stress hypothesis) (66, 76, 104). Discontinuous and cyclic respiratory patterns have been reported in a variety of cockroach species including *Blaberus giganteus*, *B. germanica*, *Macropanesthia rhinoceros*, *P. americana*, and *Perisphaeria* spp. (2). Recent reports on respiratory cycling in *Nauphoeta cinerea* indicate that they respire discontinuously to reduce respiratory water loss (119), enabling them to survive food and water restriction (118). Matthews & White (77) provide insight on factors that are involved in the control of *N. cinerea* respiratory cycling. They monitored hemolymph pH (micro-pH electrodes), VCO_2, and abdominal ventilation movements during exposure of *N. cinerea* to normoxic, hypoxic, and hypercapnic (low CO_2) atmospheres. Exposure to these conditions resulted in continuous respiratory movements to maintain constant hemolymph pH (7.3), except when the cockroaches were subjected to hypoxic or hypercapnic conditions that resulted in hyperventilation movements (77). Based on results from decapitated insects, they also found that control of ventilation was located in central pattern generators located in the thoracic and abdominal ganglia, not in the cephalic ganglion. An earlier report on the effects of hypoxia, hypercapnia, and pH on ventilation rates in *N. cinerea* showed an elevated ambient CO_2 effect that was attributed to changes in hemolymph pH (120). The role of CO_2 in regulation of ventilation movements based on the requirement for maintenance of a stable hemolymph pH is reasonable given the metabolic activities of the intact organism.

Excretion

Water loss associated with excretory processes can be considerable and must be accounted for when assessing an insect's water balance. The components that affect water excretion include the requirement to void nontoxic components (such as processed or digested food residues), excess solutes (potentially toxic if not removed from the insect), and toxic materials (81, 94, 96, 141). The cockroach alimentary canal has been well described (10, 12, 22, 46, 137), and a variety of functions can be ascribed to it. These include digestion (inclusive of microbe-associated activities), nutrient

absorption, and excretion. Among individual species, the morphology may vary considerably. A simplified version of water movements from anterior to posterior parts of the alimentary canal includes (*a*) ingestion of food with variable water content and water uptake—as water is required for all digestive and excretory activities, digestive processes may require an initial investment of water, which may be provided by the salivary glands; (*b*) food and water storage in the crop; (*c*) food processing, which may include particle size reduction in the proventriculus; (*d*) introduction of digestive enzymes and absorption of smaller molecules, including absorption of digested materials, in the gastric caeca/midgut; (*e*) reception of filtrates and materials removed from the hemolymph by the Malpighian tubules; (*f*) modification of the digested residues mixed with the Malpighian tubule fluids and subject to modification by hindgut microbes; and (*g*) retrieval of useful materials in the rectum before the processed residues are voided. This version of water-related activities as food travels through the alimentary canal is deceptively simple but useful for making two important observations. First, the excretory system is a part of the digestive system, conveying opportunities for recycling of materials that might otherwise be lost to the insect. That is, the mixing of the Malpighian tubule filtrate with the midgut effluent as it passes into the hindgut provides an opportunity for the microbes inhabiting the hindgut to recycle/detoxify materials. Second, there are efficiency levels associated with all of these processes: unavoidable uptake (toxic and excess materials) and unavoidable losses (water and nutrients that are not retrieved before fecal elimination) (73). Water loss via the fecal pellet is related to the absorptive capacity of the rectal tissues and their ability to modify excreted materials in keeping with requirements for maintenance of the insect's homeostasis.

Ammonotelic: referring to an organism that excretes soluble ammonia as an end product of nitrogen metabolism

Uricotelic: referring to an organism that excretes uric acid and/or its salts as an end product of nitrogen metabolism

Role of nitrogen in the cockroach excretory processes. A requirement of insect nitrogen metabolism involves removing excess materials from metabolic pools by either voiding them externally or storing them in some form internally. Cockroaches excrete a variety of nitrogenous materials as a means of creating balance between their dietary intake and their physiological and metabolic needs. However, the most prominent nitrogenous waste products are ammonia and uric acid. In 1985, Cochran (19) summarized much of our knowledge regarding nitrogen excretion in cockroaches. As a group cockroaches may be classified as externally ammonotelic and uricotelic and internally uricotelic. The externally excreted nitrogenous materials are ammonia and urates, whereas those stored internally are precipitated urates located either in specialized structures within cockroach fat body (urate) cells (19) or in the accessory glands of some cockroach males (108). Ammonia usually represents the major externally excreted nitrogenous waste material, except for in a few species that excrete urates externally, via the Malpighian tubules (19, 65). Most species examined so far excrete ammonia externally, and because of the relative toxicity of ammonia, this requires increased water excretion reflected by increases in water uptake. For example, in *P. americana*, a threefold increase in dietary nitrogen resulted in a 92% increase in water intake (80).

Urates play a central role in cockroach physiology and appear to be integrated components in their nitrogen metabolism (19, 100) (**Figure 2**). Typically, fat body contains three distinct cell types: (*a*) trophocytes that function as centers of intermediary metabolism and storage, (*b*) urocytes that contain stored urates as distinct crystalline spherules, and (*c*) mycetocytes that contain symbiotic bacteroids. The mycetocytes usually are found centrally within the fat body lobes surrounded by urocytes and are implicated in urate metabolism (20, 100, 144). Urate levels fluctuate in relation to the dietary nitrogen on which cockroaches are maintained, increasing in response to feeding on a high-nitrogen diet and decreasing in response to low-nitrogen diets. Circumstantial evidence, based on observed declines in body urate levels of cockroaches maintained on low-dietary-nitrogen regimens, strongly implicates a symbiont contribution to nitrogen metabolism

(19, 100). This hypothesis is supported by a variety of studies using radiolabeled uric acid (as ^{14}C-hypoxanthine, which is rapidly converted to uric acid) and demonstrating that insects on low-nitrogen diets degrade stored urates to $^{14}CO_2$ (94, 100). However, these studies do not provide indisputable evidence of the introduction of metabolized urate nitrogen into metabolic pools.

Ion sink hypothesis. Processes involved in maintenance of insect homeostasis are dependent on activities associated with their water balance, including the requirement to maintain their hemolymph osmolality, determined by solute concentrations (inorganic and organic molecules). In 1970 Wall (136) reported that *P. americana* hemolymph osmolality was strongly regulated during dehydration/rehydration cycles without exogenous inputs (food sources). She suggested that the apparent sequestration/release of Na^+ was associated with yet to be identified tissues. Her findings led Mullins & Cochran (82) to suggest that stored urates might provide such an ion sink, allowing for sequestration/release of hemolymph ions as a mechanism for maintenance of homeostasis. Since that time, several studies have supported this hypothesis: Tucker (128) reported exchanges of $^{22}Na^+$, but not $^{36}Cl^-$, with adult *P. americana* fat body tissues during hydration/dehydration; Hyatt & Marshall (53) found that both K^+ and Na^+ were sequestered/released from fat bodies during dehydration/rehydration, and by using X-ray microanalysis, they confirmed that urate crystals within urate cells sequestered both K^+ and Na^+ in water-deprived *P. americana* (54). In addition, these changes appear to be under neurohormonal control (122). It is unclear what roles cations (Cl^-, HCO_3^-, $H_2PO_4^-$, and others) might play in achieving a required electrolyte balance. Further examination of this hypothesis could be an interesting area of study because of the role that urate stores may play in maintenance of cockroach hemolymph homeostasis.

TEMPERATURE ACCLIMATION

As referenced in the introductory Overview section, cockroaches occur in virtually all habitats, including tropical and temperate forests, grasslands, heath, salt marshes, coastal communities, and deserts, with their attendant differences in temperatures (9, 107). The diversity of habitats in which cockroaches are found reflects their ability to adjust to environmental variables. This adjustment can be achieved by behavioral and physiological adaptations to habitat selection. Behavioral adaptations to temperature can involve avoiding extreme temperatures by movement to less extreme environments and physiological responses (regulation of water balance by modification of cuticular structures or respiratory patterns) or changes in heat or cold tolerance.

Heat Tolerance

Early work on temperature preferences of cockroaches under laboratory conditions has been summarized (8, 22, 46, 107). For example, the preferred temperature for *P. americana* ranges between 24°C and 33°C, but it is quite motile at temperatures of 15–32°C, becomes highly active at 34°C, and succumbs to heat paralysis at 42°C (107). Little new information is available on whole-animal responses to temperature; however, Appel (1) has provided comparative information on several species. He examined water relations and thermal sensitivity in three cockroach species, *Diploptera punctata*, *Pycnoscelus surinamensis*, and *Blatta lateralis*, noting changes in their total water content, cuticular permeability (CP), critical thermal maxima (CTMax), and upper lethal limits (ULLs). He observed several significant changes. Among these, the CP was not related to initial mass and ranged from 20.9 to 38.7 $\mu g/cm^2/h/mm$ Hg for *D. punctata* and *P. surinamensis*, respectively; there were small but significant changes in CTMax in all three species, ranging from 43.2°C for *D. punctata* to 44.3°C for *P. surinamensis*; and the ULLs were 2.2°C to approximately 4.0°C

greater than the CTMax, the greatest being 48.1°C, for *B. lateralis*, and the lowest 45°C, for *D. punctata*. Observations on desert-dwelling cockroaches indicate they exhibit morphological, behavioral, and physiological adaptations for avoiding or tolerating extreme temperatures (9). Edney et al. (34) studied *A. investigata*'s response to the desert microclimate. Their measurements of soil temperature during 24-h cycles in July (the hottest and driest month) and November (the coolest month) were correlated with vertical movements of *A. investigata* in the soil (−12.5 cm in July and −5 cm in November). They also noted that *A. investigata* in July were found at 30–35°C, whereas in November they were most frequently located at 22–26°C, suggesting that these seasonal distribution differences might be attributable to acclimation. In studies comparing the responses to microclimates of two species of *Arenivaga* (*A. investigata* and *A. apacha*), Cohen & Cohen (21) found differences in acclimation/adaptation among species: *A. investigata* has a higher tolerance and lower water-loss rates and oxygen consumption than *A. apacha*.

Cold Tolerance

Insects employ a variety of strategies in response to low temperatures, allowing them to survive exposure to cold. These include (*a*) avoiding exposure to subzero temperature (migration to warmer climates and burrowing underground), (*b*) freeze avoidance involving deep supercooling of body fluids by increases in sugar alcohols or antifreeze proteins, (*c*) freeze tolerance involving the regulated freezing of body water in extracellular spaces that is triggered by specific ice-nucleating agents or proteins, (*d*) cryoprotectant dehydration, a recently described strategy, where there is a combination of extreme dehydration with high cryoprotectant levels that results in stabilizing macromolecules, and (*e*) vitrification involving the transition of a liquid to a solid without ice crystal formation, allowing larvae to survive exposure to −100°C (125). All of these strategies have an energetic cost associated with them (9, 125). Several cold hardiness strategies in cockroaches have been reported. Freeze avoidance is achieved by microhabitat selection in *Periplaneta japonica*, which also displays freeze tolerance (126), and by acclimation in *Blatta orientalis*. Cold hardiness increased in both species when they were maintained at 10°C for up to 14 days (101). Other freeze-tolerant species, such as *Celatoblatta quinquemaculata*, utilize ice active proteins and cryoprotectants (glycerol and trehalose) (142, 143, 146). Hamilton et al. (47) reported that the hemolymph of winter-acclimated wood cockroaches (*Cryptocercus punctulatus*) contains ice-nucleating agents and that these wood cockroaches accumulate the polyol ribitol during winter. Previous work on heat and cold tolerance and water balance has provided some useful information on water management as well as maintenance of homeostasis in cockroaches. However, with the advances in new techniques and protocols, efforts to obtain better resolution of dehydration/hydration activities could allow for a more detailed understanding of their biochemistry and physiology. For example, employment of a newer technique (use of a temperature-controlled arena coupled with digital camera recording to observe behavior during cooling and warming) for rapid measurement of thermal tolerance traits, proposed by Hazell et al. (49), combined with thermal ramping assays, proposed by Overgaard et al. (99), should provide better resolution in (re)visiting low-temperature thresholds and the gradation of the development of chill comas (48). Finally, molecular techniques for examination of genes that provide for desiccation tolerance and cold tolerance may be used as a basis for understanding aquaporins that provide channels for water and small solutes to pass through cell membranes (18).

MICROBIAL PHYSIOLOGICAL AND BIOCHEMICAL CONTRIBUTIONS

Insect diversity is reflected by the large and varied microbial communities that inhabit their digestive tracts, and several studies have focused on the nutritional contributions of symbiotic gut

microbes in insects that feed on suboptimal diets (25, 29). Because gut microbes may play an important role in compensating for dietary nitrogen deficiencies in arthropods, Nardi et al. (86) suggested that microbial gut symbionts may contribute significantly to nitrogen fixation in terrestrial ecosystems. Some surmise that the early cockroach ancestors fed on detritus that was abundant during the Upper Carboniferous (19, 32, 85) and that, in turn, may have influenced the evolution of nitrogen-efficient metabolism in cockroaches and termites. Development of an efficient nitrogen economy includes the dynamics of resident gut microbes coupled with the recycling capabilities of the excretory system and the endosymbiotic microbes contained in mycetocytes.

Gut Microbes

The cockroach hindgut is adapted to harbor and retain microbial populations (14). Microspines (20–100 μm) line the entire length of the hindgut and are associated with bacterial aggregations or plaques in the ileum of at least four species (35). The relative numbers of microbial forms may vary in different hindgut regions of *Eublaberus posticus* (24). The employment of molecular technology has allowed for much more precision in identification, and hence a better understanding, of the types and relationships of the gut microbial populations. This progress has led to reports on the relatedness of cockroach and termite gut symbionts (61, 97, 117), the commonality of antifungal defense strategies in *C. punctulatus* and termites (17), and the gene diversity of [FeFe] hydrogenase genes in five dictyopteran families (5). Understanding of hindgut digestive activities in cockroaches is somewhat limited, but progress is being made. Known products of gut microbial activities include acetate produced by pathways that may involve anaerobic carbon monoxide dehydrogenase in lower termites and *C. punctulatus* (75), formyltetrahydrofolate synthetases in *C. punctulatus* and *P. americana* (98), cellulose and hemicellulose degradation (11), and production of organic acids and methane in *P. americana* hindguts (60). Absorption of small molecules (free fatty acids such as acetate and butyrate) can occur in the hindgut lumen in *Panesthia cribrata* (50) and *P. americana* (15, 151). Insect cellulolytic systems have been reviewed recently (139), providing a comprehensive report on the processes involved in rendering lignocellulosic materials into a useful metabolic form.

The recognition that ammonia may constitute a major excretory nitrogenous product in terrestrial insects (ammoniotely) has led to consideration of new hypotheses on the mechanisms involved in its transport (141). Generation of an ammonia flux by the Malpighian tubules destined for release into the anterior end of the hindgut produces an opportunity to recycle metabolic nitrogen by gut microbial systems before excretion in feces. The involvement of gut microbial systems in ammonia metabolism, coupled with subsequent absorption of potentially useful materials achieved by processes within the hindgut, is a much-needed area of investigation.

Mycetocytes

Mycetocytes, or Blochmann bodies, are specialized fat body cells first observed in 1889 in *B. germanica* and *Doryphora* [now *Leptinotarsa*] *decemlineata* by William Wheeler (64). The bacteroids contained in mycetocytes have been a subject of interest for an extended period of time (19, 28, 79). These mutualistic symbionts (*Blattabacterium* spp.) in cockroaches and termites are estimated to have appeared 135–300 Mya (79). Early work provided information on their classification (16, 64), arrangement, and cytology within the fat body (20, 78, 115) and ovaries (40, 78, 114), as well as transmission between generations via transovarial transmission (9, 113). With the exception of the phylogenetically divergent genus *Nocticola* (69), all cockroach species have mycetocytes, and among termites only the basal termite (*Mastotermes darwiniensis*) possesses them

(7, 23), leading to the suggestion that the loss of the mycetocyte relationship must have occurred during the evolution of termite lineages except for *M. darwiniensis* (7).

Mycetocyte contributions to cockroach metabolism have been implicated based on their relative abundance and apparent activity during oocyte development and molting cycles (44, 78), cytological responses to dietary nitrogen levels (20, 100), increases in fat body urates when cockroaches are fed antibiotics (130), and uric acid metabolism by mycetocyte bacteroids (27). Employment of molecular technology has revealed (*a*) that these symbionts are flavobacteria (*Blattabacterium* spp.) (6); (*b*) that nuclear DNA isolated from fat body and egg symbionts is identical (148); and (*c*) that they display cocladogenesis with their cockroach hosts (67), and this cocladogenesis may be used for tracing the history of geographic movements of cockroach populations (74). More recent molecular studies have focused on examining nitrogen metabolic capabilities of these symbionts (41, 51, 70, 87, 111, 112). Based on the circumstantial evidence that urate nitrogen is mobilized by these symbionts, the question is how this might be achieved. In order to mobilize the stored urates, some type of uricolytic activity must initiate the process. Sabree et al. (112) have found that sequences of the *P. americana*'s *Blattabacterium* genome did not contain genes capable of performing the uricolytic task but are capable of recycling ammonia and urea as potential uric acid degradation products that can be converted into useful nitrogen-containing molecules.

Early contributions to our understanding of the biosynthetic pathway for uric acid in cockroaches, including the conversion of its immediate intermediates hypoxanthine and xanthine mediated by xanthine dehydrogenase (XDH), have been reviewed (19). Wren & Cochran (147) reported that crude extracts of mycetocyte preparations from fully formed *P. americana* oothecae exhibited XDH activity in an anaerobic environment and that those from *B. germanica* oothecae exhibited aerobic XDH activity (36, 37). These results raise the possibility of an undefined pathway that could be explored by further purification and characterization of XDH from mycetocytes devoid of influences from fat body cells that do exhibit aerobic XDH activity.

Studies on the metabolism of urate nitrogen should acknowledge that the nitrogen pathway may be quite different from that of carbon. The use of radiolabeled (^{14}C) materials to trace metabolism of urate precursors and its metabolites provides only circumstantial evidence regarding the path of nitrogen. The stable isotope ^{15}N has been used with uric acid to obtain more definitive information in the past, but this approach has been limited because of the expense associated with the analysis. Potrikus & Breznak (103) demonstrated that injected ^{15}N–uric acid was incorporated into ^{15}N-labeled termite tissues, and Mullins et al. (83) found that four ^{15}N–amino acids (alanine, proline, glutamic acid, and aspartic acid) were incorporated into oothecae when *B. germanica* females were fed ^{15}N–uric acid. To examine the metabolism of soil-feeding termites (*Cubitermes* spp.), Ngugi et al. (89, 90) have used a ^{15}N-based approach involving nitrogen mineralization, denitrification, and nitrate ammonification using gas chromatography combined with isotope ratio mass spectrometry (GC-IRMS). New approaches using ^{15}N-labeled materials coupled with microanalytical tools [GC-IRMS and/or high performance liquid chromatography (HPLC)-IRMS] and other techniques might provide a better understanding of the complexities of these symbiotic relationships. Clearly, there is much to be learned about the metabolism of nitrogen. The role of gut microbes and mycetocyte symbionts in cockroaches is basic to the unique physiology and biochemistry of this group of insects.

RESOURCE MANAGEMENT AND REPRODUCTION STRATEGIES

Maternal Investment

Reproductive strategies among the Blattaria are quite diverse, representing three general categories of egg formation and encapsulation and embryo nurturing (9): (*a*) oviparity, characterized

as the production of an egg case (ootheca) that may be oviposited soon after formation or carried externally until or just before hatching; (*b*) ovoviviparity, which includes two subgroups in which either (*i*) newly formed oothecae are extended during formation but then are retracted into a brood sac, remaining there until hatching, or (*ii*) no oothecae are formed and the eggs pass directly into a brood sac; and (*c*) viviparity, where once eggs are formed they are retracted into the brood sac, where the embryos are provided with nourishment (9). These different strategies demonstrate approaches that mirror adaptations to accommodate their requirements. With respect to water balance, the oviparous oothecae deposited soon after formation are adapted to minimize water losses, whereas those oviparous oothecae remaining attached to the female may be provisioned with water from the female (84). The complexity of the respiratory arrangement adapted to minimizing respiratory water loss in eggs has been recently examined, and some of the genes involved in constructing the chorion have been identified (57–59). Obviously, in ovoviviparous and viviparous cockroaches, embryos share their water balance systems with their mothers. Investment of materials (such as vitellins) during oogenesis has been examined in a number of species and is similar to that exhibited by insects with a panoistic or ancestral type of egg development. Two additional activities occur during this process: (*a*) the required vertical transovarian transmission of *Blattabacterium* symbionts, described by several researchers (44, 115), and (*b*) inclusion of urates into the eggs (83). One of the more unusual reproductive processes is exhibited by the sole viviparous cockroach *D. punctata*, where maternal nutrient provisioning (milk) during embryonic development has been reported (55, 124). Some of the genes that encode the milk peptides have been characterized (145). Methyl-branch hydrocarbons synthesized by the female have been identified as major components of a waxy coating encasing the developing embryos (88). In addition, Youngsteadt et al. (149) found that the major cuticular hydrocarbons were synthesized by females and incorporated into developing embryos prior to parturition.

Male Contributions

Paternal investment of nutritional resources in insects may occur before, during, or after copulation and may include (*a*) providing nourishment to females from glandular products of the male (secretions from dorsal glands, salivary secretions, spermatophores, or mating plugs), (*b*) nourishment provided by males involving food collected or captured prior to mating, and/or (*c*) sexual cannibalism, where the female eats the male (4, 127, 129). Examples of the first category, and perhaps the second category, can be found among male cockroaches that provide resources to females at mating. Some of the glandular secretions involve tergal glands. These are a variety of glands that have evolved on the dorsal abdominal surface of some male Blattaria but may serve several functions (9, 106). These functions may include production and release of pheromones and/or nutritional phagostimulant-nuptial gifts (62, 63, 92, 123). Nutrients may be transmitted to females via spermatophores and, in some cases, uric acid. Insemination is achieved by insertion of a spermatophore, consisting of sperm sacs enclosed within a proteinaceous capsule, during copulation (42, 109). In some species insertion of the spermatophore may be accompanied by urates stored in specialized male accessory glands, forming a genital plug (42, 109). After the spermatophore is emptied, the female may consume the discarded spermatophore and associated urates. When ^{14}C-hypoxanthine (rapidly converted to ^{14}C–uric acid) and ^{3}H-leucine (representing protein) were injected into *B. germanica* males maintained on two diets, a significant proportion of the isotopes [^{14}C (29–41%) and ^{3}H (40–56%)] was found in oothecae produced by females after mating (83). Schal & Bell (116) demonstrated a similar transfer of ^{14}C-urates, which were offered by males as a nuptial gift to *Xestoblatta hamata* females during mating and accumulated in developing ovaries.

They suggested that these urate gifts contributed significantly to the female's nitrogen pool and may shorten the time between mating and oviposition.

CONCLUSIONS

Although cockroaches are perceived by the general public as pests that must be eliminated from human domiciles, only a few of the many species are considered to be pests. However, because of the antiquity of their lineage, their generalized morphology, and their apparent ability to inhabit a wide variety of habitats, they are a remarkable group of insects. As a result, they have served as excellent subjects for teaching biology and as a research animal spanning many areas of biochemistry, physiology, ecology, and behavior.

Their basic design, which includes an association with two microbial systems, provides them with a unique nutritional advantage. This, along with their abilities to regulate their water balance and adapt to extreme temperatures, contributes to their designation as habitat and dietary generalists. Cockroaches evolved at a time when the bulk of their diets was low in nitrogen. However, when these early primitive omnivores encountered a food source rich in nitrogen, any excess nitrogen could be stored in their fat bodies. The basic design of the insect's excretory system was such that this stored nitrogen could then be recycled in the hindgut, where it could be metabolized with the assistance of their gut microbes to provide them with a metabolic advantage. At some point in time, mycetocytes evolved into an organized cellular structure and developed an intimate metabolic relationship with the urocyte and adipocyte fat body cells. The current working hypothesis suggests that this relationship supplements their temporal dietary deficiencies, enabling them to be more successful biochemically. Collectively, the central theme is that cockroaches (and primitive termites) have two systems that provide for their nutritional needs, the mycetocyte/urate system and the hindgut system, and these systems may function in an integrated fashion.

SUMMARY POINTS

1. The elaboration of the cockroach basic design has provided them with the ability to adapt to diverse habitats that include humid, tropical; hot, dry (arid); and frigid alpine environments.
2. The design of the cockroach digestive system, in combination with their excretory system, provides an efficient means to eliminate materials that are toxic or present in excessive concentrations, but it also provides a means to store and recycle potentially useful materials into their metabolic systems.
3. Storage of urates in specialized fat body cells and in male uricose glands allows cockroaches to use them in maintaining a positive nitrogen balance when feeding on nitrogen-deficient diets. These stored reserves can be transferred to their offspring as a form of maternal and/or paternal investment. In addition, stored urates appear to play an important role in maintaining body fluid (hemolymph) osmotic homeostasis by serving as an ion sink.
4. Cockroaches rely on two distinct symbiotic microbial systems, the fat body mycetocytes and hindgut microbes, which provide them with opportunities for recycling stored urates.
5. Cockroaches have served and still are serving as useful models for studies including insect biochemistry, physiology, behavior, and ecology.

FUTURE ISSUES

1. Experimentation should continue on the energetics and regulation activities associated with the physiological and biochemical systems that provide for metabolic and osmotic homeostasis. These types of studies done under both laboratory and field conditions can provide a better understanding of how these systems perform in a natural environment.
2. The use of new technologies should allow for more precision in physiological investigations. This could include more sensitive determinations of body fluid pH, osmolality, and ultramicro chemical procedures.
3. Major advances in molecular biology utilizing genomics and metagenomics should provide more clarity in studies of evolutionary relationships between cockroaches and termites as well as studies on immune system interactions with obligate microbes.
4. Biochemical studies utilizing stable isotopes (^{15}N) and designed to explore the nitrogen metabolic pathways associated with the cockroach and its gut microbes and mycetocytes should be helpful in defining what roles symbionts play in cockroach nitrogen metabolism. This approach would be particularly helpful in resolving some of the questions being raised by investigations of the cockroach and primitive termite symbiont genomics.

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