CATABOLIC SUBSTRATE UTILIZATION PATTERNS: VARYING HUMIDITY AND DIETARY REGIMENS IN THE AMERICAN COCKROACH *Periplaneta americana* (L.) (Dictyoptera: Blattidae)

by

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(ABSTRACT)

Changes in metabolic substrate utilization patterns resulting from exposure to varying humidity and dietary regimens were examined in the adult, male American cockroach. A series of respirometric experiments were conducted to monitor changes in respiratory quotient (RQ), oxygen quotient (QO_2), food and water consumption and body weight. Also, a series of radiolabel experiments, utilizing injected ^{14}C-glucose and ^{14}C-palmitic acid, were conducted to determine radiolabel content (after exposure to varying humidity and dietary regimens) in whole body, feces, $^{14}CO_2$, hemolymph, fat body tissue and cuticle. The results of these two series of experiments were compared to determine the overall effects of humidity and diet on catabolic substrate utilization pattern changes in the American cockroach.

In these experiments, variation in dietary regimens appeared to play a more significant role than humidity variations with respect to significant differences observed in insect RQ, QO_2, body weight and radiolabel incorporation

in whole body, $^{14}CO_2$, hemolymph, and fat body tissue. However, humidity significantly affected radiolabel incorporation in fat body tissue in the glucose experiments and $^{14}CO_2$ in the palmitic acid experiments.

RQ and QO_2 data from the respirometric experiments and $^{14}CO_2$ data from the radiolabel experiments suggested that cockroaches deprived of food, but provided water to drink, change their metabolic substrate utilization pattern from initial catabolic substrates such as carbohydrates to lipid-based substrates. Also, cockroaches deprived of either water (FO) or food and water (OO) appeared to change metabolic substrate utilization patterns toward carbohydrates, but the final carbohydrate metabolic substrates are still unknown.

Cockroaches provided water, but no food, showed a change from initial RQ values (low humidity OW = 0.92 and medium humidity OW = 0.86) to terminal RQ values (low humidity OW = 0.71 and medium humidity OW = 0.68) representative of lipid-based substrate metabolism. There was also an unexplained decrease in QO_2 (initial low humidity OW = 0.45 ul/mg/hr and terminal low humidity OW = 0.28 ul/mg/hr; initial medium humidity OW = 0.35 ul/mg/hr and terminal medium humidity OW = 0.30 ul/mg/hr). Also, cockroaches deprived of water (FO) or food and water (OO) showed an overall experimental rise in RQ values (initial low humidity FO = 0.80 and terminal low humidity FO = 0.87;

initial medium humidity FO = 0.82 and terminal medium humidity FO = 0.85: initial low humidity OO = 0.79 and terminal low humidity OO = 0.88; initial medium humidity OO = 0.75 and terminal medium humidity OO = 0.87). In addition, these same insects showed an overall experimental increase in QO_2 (initial low humidity FO = 0.34 ul/mg/hr and terminal low humidity FO = 0.43 ul/mg/hr; initial medium humidity FO = 0.45 ul/mg/hr and terminal medium humidity FO = 0.49 ul/mg/hr: initial low humidity OO = 0.37 ul/mg/hr and terminal low humidity OO = 0.53 ul/mg/hr; initial medium humidity OO = 0.32 ul/mg/hr and terminal medium humidity OO = 0.45 ul/mg/hr), and in the radiolabelled glucose study OO insects showed an almost complete cessation of $^{14}CO_2$ release at 2 days (d). Terminal body weights differed significantly among cockroaches provided with both food and water (controls = FW), only water (OW), only food (FO) and no food or water (OO). Insects deprived of either water (FO) or food and water (OO) had significantly lower terminal body weights than those insects provided either water or food and water. Whole body water content increased and dry weight decreased in insects provided only water to drink.

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1.0 - INTRODUCTION

Survival is dependent upon an organism having the ability to either cope with or adapt to changing conditions in the environment in which it lives. Adaptive physiological, biochemical and behavioral strategies are required for survival under adverse conditions such as desiccation and/or starvation. The American cockroach, Periplaneta americana (L.), is an example of an insect species which has acquired several adaptations that directly contribute to its success.

The success of this insect, in part, has been attributed to its ability to withstand extended periods of desiccation and/or starvation (Willis and Lewis, 1957; Tucker, 1977c; Hyatt and Marshall, 1985), as well as changes in ambient temperature and humidity (Gunn, 1935; Edney and McFarlane, 1974; Appel et al., 1983; Rollo and Gunderman, 1984). Considerable research relating adaptive responses of the American cockroach to environmental conditions has been conducted; studies include changes in food and water consumption patterns (Lipton and Sutherland, 1970), osmoregulation under conditions of stress (Wall, 1970; Tucker, 1977c; Heit et al., 1973) and behavioral responses to metabolic reserves (Rollo, 1984).

Some workers have suggested that the American cockroach may have the ability to regulate their metabolic patterns to supplement water and energy demands under conditions of desiccation and/or starvation (Wall, 1970 and Tucker, 1977c). Alternately, other workers have suggested that these changes in metabolic patterns in insects may be counterproductive in terms of metabolic water production compared to the energy required to provide this extra metabolic water. For example, only 0.28 $mg/g^{-1}/h^{-1}$ of metabolic water is produced in starved or desiccated locusts as compared to 0.35 $mg/g^{-1}/h^{-1}$ in fed locusts (Loveridge and Bursell, 1975). The primary sources of identified energy reserves in insects include the fat body and muscle tissue (Orr and Downer, 1982), hemolymph (Downer and Matthews, 1978) and the cuticle (Bade and Wyatt, 1962; Steele, 1983). However, an understanding and documentation of the availability and subsequent utilization patterns of metabolic reserves in response to conditions of desiccation and/or starvation in insects is lacking.

Therefore, to obtain information on changes in metabolic substrate utilization patterns in response to differing conditions of humidity and dietary availability in the American cockroach, the following studies were undertaken:

1. Examination of physiological and metabolic responses to differing humidity and dietary regimens by monitoring changes in respiratory quotient (RQ),oxygen quotient (QO_2), food and water consumption patterns and body weight.

2. The determination of changes in substrate utilization patterns in the whole insect after exposure to different humidity and dietary regimens using radiolabelled glucose and palmitic acid as substrates and monitoring radiolabel incorporation in whole body, feces, CO_2, hemolymph, fat body tissue and cuticle.

The goal of these objectives was to test the hypothesis that changes in metabolic substrate utilization pattern(s) occurred in cockroaches when exposed to varying conditions of humidity and dietary availability, and to gain a better understanding of the relationship of stress to metabolic pattern changes.

2.0 LITERATURE REVIEW

General reviews on cockroach biology are available and include Cornwell (1968), Guthrie and Tindall (1968) and Bell and Adiyodi (1981). Other reviews provide information on specific metabolic/physiological processes occurring in insects. These include energy reserve utilization and metabolism (Candy, 1981), metabolic water production (Loveridge and Bursell, 1975) and water compartmentalization and kinetics in insects (Wharton and Richards, 1978; Machin, 1981). In addition, several reviews provide information on the specific aspects of substrate metabolism such as carbohydrates (Steele, 1981), lipids (Downer, 1978; Beenakkers et al., 1981a) and nitrogen containing compounds (Mullins and Cochran, 1983).

Stress-induced changes of metabolic pathways are viewed as an attempt to maintain homeostasis with respect to energy and water balance requirements. Some workers have reported tissue ion sequesteration as a response to water deprivation (Wall, 1970; Heit et al., 1973; Tucker, 1977c; Hyatt and Marshall, 1985). A fat body ion sink in water- and diet-stressed American cockroaches has been proposed for storage of Na^+, K^+ and NH_4^+ as urate salts of

these cations (Mullins and Cochran, 1974; Tucker, 1977c). Additional support for this hypothesis has been provided (Hyatt and Marshall, 1985). Tucker (1977c) suggested that a metabolic pattern change (from initial substrates to primarily lipids) may occur in dehydrating cockroaches in an attempt to supplement dwindling water reserves as water is removed from the hemolymph.

The available literature has helped provide an understanding of common metabolic pathways and variations which occur in an attempt to maintain energy and water balances when insects are physiologically or environmentally stressed. The following discussion of various environmental and physiological conditions affecting metabolic and biochemical homeostatic processes is not intended to be comprehensive, but rather to summarize the information now available regarding current hypotheses and theories of metabolic homeostasis in insects. Special attention will be given to the current information concerning these theories in the American cockroach.

2.1 - PHYSIOLOGICAL PATTERNS

It has been suggested that changes may occur in metabolic patterns, with regard to the substrates destined

for catabolism, in response to the availability of food and water (Tucker, 1977c), requirements of different tissues (Loveridge and Bursell, 1975), activity (Cooper, 1982), humidity (Arlian and Wharton, 1974) and developmental stages (Bade and Wyatt, 1962; D'Costa and Birt, 1966; Lipsitz and McFarlane, 1971; Chippendale, 1973). Several studies suggest these changes in physiological patterns are hormonally regulated (Steele, 1961; Gilbert, 1967b; Downer, 1972; Downer and Steele, 1972; Steele, 1983).

Changes in carbohydrate and lipid metabolism patterns in response to various physiological and environmental stimuli have been reported. For example, a recent study indicated that a possible stress-related, carbohydrate-to-lipid, reserve utilization pattern may occur in the American cockroach when exposed to applications of lindane (Orr and Downer, 1982). Water reserves in Rhodnius have been shown to be affected by insecticides, an effect attributed to antidiuretic hormone release (Casida and Maddrell, 1971; Maddrell and Casida, 1971). Age and nutritional state play major roles in the regulation of insect metabolic and water reserves (Riegert, 1958; Wharton et al., 1965; Loveridge, 1973; Tucker, 1977b,c). Also energy reserves are reported to be affected by diet (Municio et al., 1973) and an insufficient, energy-producing diet has been shown to cause oocyte resorption in female Periplaneta starved for 10 days (Bell, 1971).

2.1.1 - PHYSIOLOGICAL INFLUENCE OF WATER ON ENERGY RESERVES

Some workers have studied water balance in insects in response to metabolic patterns (Laird et al., 1972; Cooper, 1982). In a review, Arlian and Veselica (1979) suggested that water balance maintenance requires that the quantity of water entering and leaving the system approximately balance during some increment of time so that body water content does not fall below or rise above critical limits. Various aspects of body water content, water loss tolerances and water fluxes were discussed in this review. Insect water gain results from imbibed food, free water, metabolic water and absorption of water from the atmosphere. Insect water loss results from simple diffusion from the respiratory and general body surfaces, by secretion of digestive fluids, pheromones, reproductive products and during oviposition, defecation and excretion (Arlian and Veselica, 1979).

Evaporative water loss in insects has been extensively studied (Ramsey, 1935; Edney, 1968; Heit et al., 1973; Browne and Van Gerwen, 1976; Hyatt and Marshall, 1977; Tucker, 1977c; Sigal and Arlian, 1982; Cooper, 1983). Integumentary transpiration and spiracular water loss

appear to be major routes of water loss in some insects (Cooper, 1983). Transpiration rates are known to be affected by temperature in both Arenivaga investigata and P. americana (Edney and McFarlane, 1974). Although water vapor absorption has been described in the desert cockroach, A. investigata (O'Donnell, 1977), there is no evidence that P. americana has the ability to absorb water from the atmosphere. Treherne and Wilmer (1975) suggested in P. americana integumentary water loss is under hormonal control.

Water balance is affected by starvation and desiccation (Arbogast and Carthon, 1972). Increased water demand induces the utilization of stored reserves and metabolic water production in some stored product pests (Devine, 1978; Cooper, 1983). Cooper (1982) found that active, free-ranging tenebrionid beetles must drink to maintain water balance or absorb atmospheric water. However, inactive beetles can maintain their water balance by eating seeds or metabolizing fat reserves.

The significance of water absorbed across insect integuments from the atmosphere has been examined in the rice weevil, Sitophilus oryzae (Arlian, 1979). Experiments involving tritiated water and measurements of water vapor absorption with S. oryzae resulted in several findings. These were: 1) Insect water content was proportional to food water content and ambient water vapor concentration

(quantities of food consumed were regulated by water content of the food and ambient water vapor concentration). 2) Imbibed water and passively absorbed water (from ambient air containing various concentrations of water vapor) constituted the major avenues of water gain. 3) Absorption rates were proportional to water vapor concentrations and transpiration rates. 4) Reduced feeding (reduced water gain from diet) at high water vapor concentrations was compensated by increased absorption rates. 5) Metabolic rate was independent of water vapor concentration, and that production of metabolic water was insignificant relative to overall water exchange.

Several effects of dehydration and rehydration have been reported for P. americana. Tucker (1977a) found that during dehydration adult, male P. americana were able to osmoregulate their hemolymph. Metabolic water alone was not sufficient to maintain water balance under conditions of extreme desiccation. She reported the total percent water content in dehydrated P. americana was lower than in hydrated individuals (cockroaches dehydrated for 12 days lost about 30% of their original weight).

Machin (1981) suggested dehydration causes conflicting demands of cells and hemolymph. Stimulation of fat body reserves occurs as the blood volume decreases. Wall (1970) used 1 to 3-week-old adult, male American cockroaches and observed dehydrated insects given water to drink

continuously. Their weights fluctuated from day to day, but did not decline significantly over a 16 d period. In contrast, insects undergoing dehydration showed an average weight loss of 10-12 mg/insect/day.

Reduced hemolymph volume due to prolonged dehydration of P. americana nymphs prevents hemolymph sampling and upon inspection of the hemocoel little hemolymph is apparent (Edney, 1968; Wall, 1970; Tucker, 1977a). Wall (1970) estimated the blood volume for cockroaches dehydrated for 8 d to be 53 ul $\pm$ 5.3 (SEM), whereas normal adult males had 130 ul $\pm$ 6.3 (SEM). Rehydration after 8 d of dehydration produced a blood volume of 164 ul $\pm$ 12.2 (SEM) and weights returned to the same level as observed on day 1 of the experiment.

When water loss from an insect is greater than that amount of metabolic water obtained by catabolism of stored foods, water is removed from the hemolymph to maintain water balance in the tissues (Tucker, 1977a). Hyatt and Marshall (1985) suggested that prolonged dehydration conditions result in a decrease in water content of the tissues (cockroaches dehydrated for 8 d lost nearly 50% of their hemolymph volume and approximately 25% of their tissue water). Tucker (1977a) suggested that after 13 d of dehydration the tissues may be so stressed that normal functions are disrupted.

2.1.2 - EFFECTS OF TEMPERATURE AND HUMIDITY

Temperature and humidity affect physiological processes in cockroaches. Water loss and respiratory rates increase as temperatures increase in dry air. Recently, information regarding cuticular permeability and critical thermal maxima corresponding to differing habitat humidities and habitat temperatures in cockroaches have been reevaluated. Early studies (Cornwell, 1968; Guthrie and Tindall, 1968) associated temperature and humidity in the context of xeric, mesic or hygric habitats and did not consider temperature and humidity independently. There appears to be no association between cuticular permeability and critical thermal maxima as had been previously reported. Cuticular permeability appears to be related more to habitat moisture than to temperature (Appel et al., 1983).

Temperature has been shown to affect food consumption and reproduction in cockroaches. For example, decreases in temperature from 25°C to 20°C may more than double the length of the reproductive cycle in female P. americana, but does not affect the amount of food accumulated during the inter-ovipositional intervals or the size of the oothecae. The effect of ambient temperature appears to be directly related to alterations of basal metabolic rates.

In addition, it is suggested that food consumption is controlled partially by metabolic demand (Rollo and Gunderman, 1984) .

2.1.3 - PHYSIOLOGICAL INFLUENCE OF ACTIVITY

Increased activity or excitement appear to affect metabolic substrate availability (Matthews and Downer, 1974; Matthews et al., 1976). Conflicting results have been reported regarding studies designed to examine physical activity levels in response to various stimuli. For example, Reynierse et al. (1972) reported reduced activity in P. americana when faced with desiccation and starvation. They suggested that reduced body weight resulted from a combination of water loss and metabolism of stored energy reserves. Rollo (1984) also reported a slight reduction in activity associated with starvation, but observed that overall activity was relatively unchanged by either starvation or sugar feeding.

Fuchs et al. (1982) reported on the response of activity and circadian rhythm to temperature and humidity. They found that exposure of Blatta orientalis to high humidity and high temperature resulted in a 1000% increase in activity, but exposure to low humidity and high temperatures resulted in a 250% increase in activity in

this species. They suggested that warm, moist stationary air promoted activity because effects of evaporation and air convection are considerably reduced. They also found that there was a complete loss of diurnal rhythm which occurred only under conditions when low temperature (15 ^{o}C) and high humidity (80% R.H.) are combined. A decrease in activity occurred only when exposed to a combination of low temperature (15 ^{o}C) and 30% R.H.

2.2 - STORAGE RESERVOIRS OF ENERGY RESERVES

Virtually every insect tissue can be considered an energy reservoir because of the presence of stored cellular glycogen (Downer, 1983). Insects have been shown to possess several sites for storage of metabolic reserves. These sites and corresponding patterns of reserve utilization often are dependent on the developmental stage and the nutritional state of the insect. The primary reservoir site is the fat body (Siakotos, 1960; Beenakkers et al., 1981b; Steele, 1983). The tsetse fly, Glossina morsitans has been shown to rely on fat body lipid reserves until the first blood meal can be obtained (D'Costa and Rutesasira, 1973). Hemolymph has been identified as a site of metabolic reserve containing trehalose, glucose and lipids (Downer and Matthews, 1976, 1977 and 1978;

Chippendale, 1978; Van der Horst et al., 1978). The insect cuticle has been suggested as a carbohydrate energy reservoir during periods of starvation (Steele, 1983). Flight muscle and intestine have been demonstrated to contain trehalose which can be utilized as an energy source (Gilby et al., 1967; Friedman, 1985).

2.2.1 - CARBOHYDRATE ENERGY RESERVES

Current reviews on insect carbohydrate metabolism have been provided (Storey and Bailey, 1978a; Friedman, 1983; Steele, 1983). Carbohydrates have been recognized as primary sources of energy production and structural components in insects. Virtually all insect tissues contain or have access to carbohydrates via hemolymph circulation. Glucose is stored in insects as hemolymph trehalose and intracellular glycogen granules. Chitin (a polymer of acetylated glucosamine residues) is the basic structural component of the insect cuticle and contains carbohydrate reserves which are shuttled back and forth between cuticle and fat body (trehalose, hemolymph sugar, is the primary vehicle of transport) (Steele, 1983).

2.2.1.1 - TREHALOSE

The major function of trehalose has been reported to be an energy source used by tissues bathed in the open circulatory system. Trehalose (alpha-D-glucopyranosyl-alpha-D- glucopyranoside, a nonreducing disaccharide) is known to be an essential source of energy in several biochemical processes such as flight metabolism, protein synthesis, fat body metabolism and water transport across the gut wall (Friedman, 1983). Insect hemolymph trehalose concentrations may range from 250 ng/ul in the tsetse fly, Glossina morsitans (Geigy et al., 1959) to more than 70 ug/ul in the female adult aphid, Megoura viciae (Ehrhardt, 1962).

2.2.1.1.1 - TREHALOSE METABOLISM

Insect trehalose levels are regulated by synthesis and storage of glucose and other carbohydrates. Metabolic demands affect the biochemical fate of hemolymph trehalose in insects and it is mobilized by hemolymph trehalase via hydrolysis of trehalose to glucose. Therefore, energy from carbohydrate reserves is made available to all insect tissues (Friedman, 1983). Trehalase activity also has been demonstrated in fat body tissue in P. americana (Storey and

Bailey, 1978a), male accessory glands (Takahashi et al., 1980) and flight muscle and gut wall (Friedman,1983).

Trehalose levels are regulated primarily by phosphorylases which have been shown to be the most important regulatory units in the trehalose biosynthetic pathway. A feedback mechanism also has been described by which trehalose metabolism is regulated according to concentrations of trehalose present and by biochemical demand (Steele, 1983). Trehalose efflux from P. americana fat body (major site of synthesis) is stimulated in vitro by corpora cardiaca extracts and is absolutely dependent on Ca^{2+} in the medium bathing the tissue (McClure and Steele, 1981; Steele and Hall, 1985; Steele and Paul, 1985). Gole and Downer (1979) suggested octopamine and trehalagon (hypertrehalosemic hormone) both share a common role in primary stimulation of trehalose synthesis in the fat body of P. americana. In vitro studies have shown that both octopamine and trehalagon stimulate AMP synthesis (AMP = second messenger) which acts to transform phosphorylase b (inactive form) to the phosphorylase a (active form) by means of a protein kinase. This in turn stimulates the utilization of glycogen reserves, resulting in the release of trehalose from the fat body into the hemolymph. Steel (1961) reported that a hypertrehalosemic factor, responsible for elevated trehalose levels in the hemolymph, increased the activity of the fat body phosphorylase.

Downer (1979) has shown that an excitation-induced hypertrehalosemic (EXIT) response in P. americana occurred as a result of treatment with several neurotransmitters.

2.2.2 - GLYCOGEN RESERVES

Glycogen is the major polymeric storage form of glucose and has been shown to be present in virtually every insect tissue examined. It is stored in large amounts in the flight muscles of insects where it may serve as an immediate glucose source for flight energy. These reserves might be quite significant, for example, in P. americana flight muscle where a concentration of 91 M/g wet weight has been reported (Downer and Parker, 1979).

The fat body also is capable of storing large amounts of glycogen. Frequently glycogen is stored in the mycetocytes of the fat body (Wuest, 1978). Fat body trophocytes and oenocytes contain small fields of stored glycogen (de Loof and Lagasse, 1970). These reserves may provide glucose to other tissues (via trehalose) (Friedman, 1985). Relative amounts of glycogen may vary between species, particularly with regard to the age and nutritional state of an insect (Beenakkers et al, 1985).

2.2.2.1 - GLYCOGEN METABOLISM

Glycogen is converted to glucose by glycogen phosphorylase before metabolism by glycolytic or other biochemical pathways. This enzyme may be hormonally activated and has a requirement for Mg^{2+}. The levels of glycogen utilization in flight muscle may be quite variable. For example, glycogen is reduced 64% in the flight muscle of Schistocerca gregaria 10 seconds after the initiation of flight (Rowan and Newsholme, 1979) and 75% in Locusta migratoria fat body after 2 hr of flight (Van Marrewijk et al., 1980).

Glycogen phosphorylase appears to exist in 2 forms. These forms have been shown to be either phosphorylase a or phosphorylase b (active and inactive forms, respectively). In Phormia regina, the modulation between the inactive and active forms has been shown to occur by AMP activation of a phosphorylase kinase (Sacktor et al., 1974) and the return to the b form via a phosphorylase phosphatase (Childress and Sacktor, 1970). In P. americana fat body glycogen phosphorylase has been shown to be significantly affected by Ca^{2+} concentration (McClure and Steele, 1981). P. americana fat body phosphorylase activation is proposed to be under hormonal control (Chino, 1985).

A "hypertrehalosemic" hormone has been shown to elevate the trehalose levels in the hemolymph of P.

americana (Steele, 1961) and reduce the glycogen in the fat body of Blaberus discoidalis (Ralph, 1962; Bowers and Friedman, 1963). These events have been attributed to interactions of the corpora cardiaca factor and the fat body (Steele, 1963). The method whereby the hormone activates the phosphorylase is not completely understood (Stone and Mordue, 1980) and the precise physiological role of these factor(s) is unknown (Friedman, 1985).

2.2.3 - LIPID ENERGY RESERVES

Lipids in the forms of monoacylglycerols (MG), diacylglycerols (DG) and triacylglycerols (TG) have been shown to play several significant roles in insects. The predominant lipid class in insects has been identified as TG which serves as a reserve of metabolic energy that can be mobilized and utilized in response to bioenergetic demands (Beenakkers, 1983).

Utilization of lipids as an energy source has several advantages. These are: 1) They have a higher caloric content/unit weight. 2) When oxidized, 2 times more metabolic water may be formed than that formed from the same weight of carbohydrate. 3) They may be stored in anhydrous form whereas glycogen is stored in a "bulky" hydrated form (Downer, 1985).

Lipids tend to be the major source of metabolic energy reserve in insects which undergo prolonged periods of metabolic activity without feeding and during nonfeeding stages (Downer, 1983). It has been suggested that insects may increase their lipid utilization rate during periods of desiccation and/or starvation (Gilbert, 1967a; Downer and Matthews, 1976; Downer 1978; Beenakkers et al., 1981a).

Absolute amounts of TG within individual insects have been shown to vary according to patterns of bioenergetic flow, in response to temperature, degree of excitation, nutritional state and circadian rhythmicity (Downer, 1981a). The major site of TG storage in P. americana is reported to be in the adipocytes of the fat body (Keeley, 1985) and may account for over 50% of the wet weight (Downer, 1981b).

2.2.3.1 - HEMOLYMPH LIPIDS

The occurrence of lipids in the hemolymph of some insects has been well documented (Chippendale, 1971). However, the predominant form of lipid in the insect hemolymph is DG bound by the carrier proteins, lipophorins or diacyllipoproteins (Gilbert and Chino, 1974; Downer and Matthews, 1976; Chino et al., 1981b; Van der Horst et al., 1981a,b; Chino and Downer, 1982).

The turnover of hemolymph DG has been studied in several species under various experimental conditions. The results indicated increased turnover occurred with increased metabolic demand (Van Handel and Nayar, 1972; Van der Horst et al., 1978; Langley et al., 1981).

The acyl groups of DG in the hemolymph in P. americana have been shown to be different from those in TG found in the fat body which suggests that specific DG synthesis occurs in an active compartment in the fat body prior to DG release into the hemolymph (Beenakkers et al., 1985). A lipoprotein lipase in the hemolymph of P. americana which could facilitate this conversion cannot be discounted (Downer, 1983). Neutral lipids in P. americana are transported in the hemolymph bound to lipoproteins (diacylglycerol lipoproteins = DGLP) synthesized by the fat body (Downer and Chino, 1979).

2.2.3.2 - FAT BODY LIPIDS

P. americana fat body has been described as a major storage site for dietary lipids (Beenakkers et al., 1985). The fat body has been shown to be important in fatty acid synthesis as demonstrated by incubation studies with fat body (Zebe and McShan, 1959; Clements, 1959; Tietz, 1961). A monoacylglycerol transferase has been reported in the fat

body of P. americana (Hoffman and Downer, 1979a). Diacylglycerides have been shown to be the primary products of lipolysis in incubated fat body homogenates of P. americana (Hoffman and Downer, 1979b). It has been proposed that, in insects, under conditions of high energy demand (i.e. locust and moth) result in the activation of a TG- specific lipase. This lipase would produce free fatty acid and DG (in the fat body) which are then released into the hemolymph (Downer, 1983).

2.2.3.3 - CUTICULAR LIPIDS

Lipids play an essential role in the formation and function of the insect cuticle (Bloomquist et al., 1982). Some cuticular lipids have been shown to originate from hemolymph lipoproteins of the American cockroach (Chino et al., 1981a). Some fatty acids liberated by lipolysis of TG reserves may serve as precursors for synthesis of cuticular hydrocarbons and be incorporated into long-chain cuticular hydrocarbons. This process is hypothesized to be in response to desiccating conditions in some instances (Dwyer et al., 1981). These cuticular hydrocarbons are suggested to be possible substrates and products of lipid metabolism (Downer, 1985).

Insects secrete a thin layer of lipid on the outer cuticular surface as a protection against desiccation, abrasion and penetration by microorganisms. The composition of this cuticular lipid fraction is variable. Cuticular hydrocarbons in some insect species account for more than 90% of the total lipids and in others primary alcohols comprise more than 70% (Bloomquist and Jackson, 1973; Downer, 1985).

2.2.3.4 - OTHER LIPIDS

Phospholipids are known to be an essential component of biological membranes and are distributed ubiquitously in all insect tissues. Total phospholipid composition of lipid extracts from many insect species are summarized by Fast (1964, 1971) and Bridges (1972). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) have been identified as the major phospholipids in insects and account for over 70% of the total phospholipid complement in most species examined (Downer, 1983).

Sterols are required in insect diets due to the inability of insects to synthesize steroid nuclei, specifically the cyclopentanoperhydrophenthrene ring (Svoboda and Thompson, 1985). Sterols serve as hormones and are important structural components of biological

membranes, are constituents of the cuticular lipid layer and are present in hemolymph plasma both in free as well as esterfied forms (Downer and Chino, 1979). In addition, sterols have been shown to modulate physical properties of phospholipid bilayers and influence membrane function (Oldfield and Chapman, 1971; Downer, 1985).

2.3 - LIPID METABOLISM

Various reviews have been published on lipid biochemistry. These include Fast, 1964; 1971; Downer, 1978; Beenakkers et al., 1981a,b; Chino and Downer, 1982; Beenakkers et al., 1985; Downer, 1985. Most potential metabolic energy available from TG is contained within the fatty acid component of the molecule. The major proportion of fatty acid complement in insects is represented by 8 fatty acids. These have been identified and include the saturated fatty acids (myristic acid, palmitic acid and stearic acid), the monounsaturated fatty acids (myristoleic acid, palmitoelic acid and oleic acid) and the polyunsaturated fatty acids (linoleic acid and linolenic acid) (Downer, 1983).

Qualitative and quantitative insect lipid pools are influenced by the nature and amount of dietary carbohydrate (Moore, 1980). The interaction between dietary

carbohydrate and lipogenesis is likely related to the activity of the pentose phosphate cycle (Geere and Perille, 1977) and may be influenced by the rate of lipogenesis (Geere et al., 1979,1981).

2.3.1 - HORMONAL CONTROL OF LIPID METABOLISM

Activities relying heavily on lipids lead to rapid changes in lipid distribution in insects. Lipid pools are known to change with time and remain in balance as long as the changes are subject to regulatory mechanisms. A number of hormones have been identified and are known to affect lipid metabolism, possibly indirectly (i.e. ecdysone has been shown to influence lipoidal components in cellular membranes while exerting its direct effect on molting and metamorphosis). Some insect hormones and pheromones have been reported to be lipid-like in nature (Beenakkers, 1983). Some of these will be considered below.

2.3.1.1 - ADIPOKINETIC HORMONE (AKH)

Mayer and Candy (1969a) demonstrated that aqueous extracts of corpora cardiaca (CC) injected into Schistocerca gregaria produced a 3-4 fold rise in hemolymph

elevated levels found during flight suggesting the presence of an "adipokinetic" factor in CC. The presence of adipokinetic hormone (AKH) has been demonstrated to occur in P. americana CC (Goldsworthy et al., 1972). Stored fat body lipids (TG) undergo lipolytic activity, via AKH, in order to synthesize necessary DG (Lok and Van der Horst, 1980; Tietz and Weintraub, 1978). The mechanism by which the release of AKH is triggered remains undetermined, although several studies indicate that circulating levels of metabolites may influence this event (Downer, 1985).

AKH has been shown to stimulate a 3-4 fold increase in cAMP in L. migratoria and S. gregaria fat body (Spencer and Candy, 1976). It has been suggested that cAMP mediates the hormonal control of DG mobilization by activating responsible enzymes by way of protein kinase(s). This mobilization may be due in part to depressed hemolymph trehalose levels and other unidentified mechanisms (one possible mechanism being the release of the biogenic amine, octopamine) (Downer, 1985).

2.3.1.2 - HYPOLIPAEMIC FACTOR

A hypolipaemic response has been reported to occur in P. americana and is accompanied by an increase in fat body diacylglycerols when cockroaches are injected with aqueous

diacylglycerols when cockroaches are injected with aqueous extracts from locust corpora cardiaca (Downer, 1972; Goldsworthy et al., 1972). The hormone(s) involved may regulate hemolymph lipid levels after feeding or hyperlipaemic episodes. This suggests facilitated regulation of transport of specific dietary lipids to the fat body for storage, thus enhancing lipogenesis (Downer, 1985).

2.3.1.3 - CORPORA ALLATA (CA) AND CORPORA CARDIACA (CC) INFLUENCES ON LIPIDS

Beenakkers et al. (1981b) have shown that CA extracts affect lipid content in locusts and that allectomy increased lipid production and resulted in hypertrophy of the fat body. A relationship has been demonstrated involving CA extracts and fat body hemolymph lipids in adult, female desert locusts. Specific TG are biochemically degraded and DG are synthesized from these TG prior to leaving the fat body. It has been shown that CA extracts affect specific DG released from the fat body. This was first reported in Hyalophora cercopia (Chino and Gilbert, 1965). Additional support for this hypothesis was reported in a study on Schistocera gregaria (Carlsen et al., 1979), which suggested a peptide with adipokinetic

activity was isolated from the corpora cardiaca of this insect. This hormone, possibly a blocked octapeptide, displays similar lipid-mobilizing properties to the original AKH (Downer, 1985).

Injected CC extracts decreased TG and DG levels in P. americana hemolymph. This hypolipaemic response is accompanied by an increase in fat body lipid. It is suggested that the responsible hormone(s) function in the regulation of hemolymph lipid levels after feeding and subsequent transport to the fat body (Downer and Steele, 1972; Downer, 1985).

2.4 - STRESS-INDUCED ALTERNATE METABOLIC ENERGY PRODUCTION

Most normal insects studied have been shown to feed periodically and in the post-absorptive state the metabolic flux tends to conserve glucose (for nerve tissue utilization, thereby favoring gluconeogenesis pathways). Non-nervous tissues appear to derive most of their energy during this state from oxidation of lipids and/or protein (Downer, 1981a). Although rapid responses to stimuli (i.e. rapid evasive action) after prolonged ($\geq$ 15 d) periods of starvation have been observed, these responses may be explained by possible metabolism of ketone bodies to

provide a major source of energy to the insect brain and nervous system after most carbohydrate and lipid reserves are depleted (Strang 1981; Downer, 1981a,b). Ketone metabolism would allow for glucose utilization in the nervous tissues and provide required energy to thoracic musculature during starvation (Shah and Bailey, 1976; Beis et al., 1980). However, the validity of the role of ketone metabolism to satisfy the metabolic requirements of the insect nervous system during starvation has not been substantiated (Strang, 1981).

Recent studies have indicated that starvation of adult cockroaches results in sequential mobilization of carbohydrates and lipids (Downer, 1983). An unpublished study by Orr and Downer suggested that adult male P. americana starved for more than 15 d exhibited an initial rise in hemolymph lipids and a corresponding decline in hemolymph trehalose, until the 9th day. At that time, lipid reserves apparently were depleted and their levels declined. Downer (1983) hypothesized from this unpublished information that a carbohydrate-lipid, feedback cycle may operate in insects. In order for the lipid portion of the feedback cycle to be activated, glucose utilization must be slowed. In mammalian muscle, citrate (increased levels are observed during fatty acid oxidation) has been shown to inhibit phosphofructokinase (the enzyme responsible for the continued utilization of glucose). Therefore, the

glycolytic pathway is slowed (Newsholme and Start, 1973). Citrate inhibition of phosphofructokinase has not been shown to occur in insects (Strang et al., 1979). It has been suggested that other glycolytic enzymes may be responsible for the inhibition of the glycolytic cycle in order to activate the lipid portion of the suggested feedback cycle (Downer, 1985).

2.5 - ENERGY PRODUCTION AND PATTERNS OF SUBSTRATE UTILIZATION

Many techniques for monitoring metabolic processes have been developed. They include respirometry (including respiratory quotient (RQ) and oxygen quotient (QO_2) determinations, biochemical assays and radiolabelled substrate utilization (Guthrie and Tindall, 1968; Richards, 1969; Aldrich et al., 1980; Steele, 1983).

An early respirometric study (Richards, 1969) on adult P. americana indicated that under conditions of complete inanition (complete deprivation of food and water) the rate of oxygen consumption decreased in a nonlinear manner independent of circadian and ultradian rhythms. It appeared clear that confinement plus complete inanition led to a general lowering of activity and/or basal metabolism. Unfortunately, this study was conducted on a

very limited number of insects. Based on the total oxygen consumption value of 140 ml for 1 of the surviving cockroaches (over an 18 d period) it was suggested this insect was metabolizing primarily lipid reserves. In addition, prostrate cockroaches showed an 8-12 fold increase in oxygen consumption prior to death.

The effects of temperature, sex and circadian rhythm on oxygen consumption have been reported in cockroaches (Banks et al., 1975). This study indicated that both Blaberus giganteus and Blatta orientalis exhibit a disproportionate increase in oxygen uptake at 36°C compared to those examined when temperature was elevated from 16°C to 26°C. In addition, after a few hours to a few days, high mortality occurred in both species, especially in the males. These workers suggested that oxygen consumption in cockroaches is subject to an endogenous daily rhythm which is dependent on both dark and light alternation periods (Banks et al., 1975).

Cervenkova (1960) reported that in starved, 2-month-old, adult P. americana, 66% of the energy used resulted from the oxidation of lipid, whereas only 22% of the energy was from glycogen and 11.8% from protein. Gourevitch (1928) found fasting cockroaches had respiratory quotients of 0.69-0.85 and suggested this was attributable to lipid catabolism. Tucker (1977c) reported RQ's for a few

dehydrated male P. americana. A mean value of 0.76 was recorded for adults and 0.67 for late instar nymphs.

Mobilization of specific energy pools (i.e. lipid, carbohydrate or protein) in insects in response to specific state(s) of hydration and/or starvation is presently not known (Tucker, 1977c). Melampy and Maynard (1937) found little difference between the lipid content of fed and starved adult male Blattella germanica. Pilewiczowna (1926) reported that during starvation for 33 days, B. orientalis lost 12% of its protein, 18% of its lipid and 70% of other non-nitrogenous substances (presumably glycogen). Nelson et al. (1967) found the hemolymph of 8th instar male cockroaches contained 83% TG and 10% DG. Young adult, cockroach hemolymph contained 36% TG and 69% DG. It has been suggested from this information that if lipid catabolism is important during dehydration, nymphs should withstand desiccation better than adults.

Changes in primary catabolic substrate utilization patterns have been suggested to occur in order to maintain some form of homeostasis with respect to insect energy metabolism and water balance. Fluctuations in basic insect metabolism are known to occur in response to certain physical factors such as insect age and sex. In addition, changes in metabolic patterns may be stress induced by starvation, desiccation, dietary availability, reduction in physical activity and isolation. All of these play major

Significant problems encountered in attempting to interpret information related to insect stress-induced metabolic pattern changes include: 1) Do changes in metabolic patterns result from interactions of two or more factors (i.e. state of hydration and dietary availability), or do changes in metabolic patterns have a sequential nature? For example, does physical isolation promote reduced physical activity, in turn reducing dietary intake, ultimately changing the basal metabolic rate? 2) Are specific changes in metabolic patterns characteristic of insect developmental stage, sex, or species, and how does each factor affect particular changes in metabolic patterns? 3) Do individual insect variations, irrespective of other considerations, make it difficult to determine specific effects for each factor involved?

Few studies have provided the tangible and unequivocal results required to interpret specific factor-response relationships. This study was undertaken in an attempt to document some stress-induced changes in metabolic patterns and to identify factors responsible. Hopefully, the following information will contribute to the general knowledge of insect metabolism.

3.0 - METHODS AND MATERIALS

3.1 - REARING AND SELECTION PROCEDURES

All studies were conducted on adult, male American cockroaches, Periplaneta americana (L.), ranging in age from 1-3 months. The insects used were obtained from the VPI & SU colonies which have been in culture for several years. Large nymphs of similar age were held together in order to maintain age groupings. The cockroaches were reared on Wayne dog chow and distilled water until adult ecdysis. Laboratory colonies were reared in 5 gal aquaria with fiberboard spacers, provided with Wayne dog chow and water and maintained on a 12 h dark/12 h light photoperiod at 25 ^{o}C. Newly-emerged, adult males of similar age were placed together in battery jars and fed the same diet as the nymphs until initiation of each experiment.

3.2 - EXPERIMENTAL PROTOCOL

Two separate studies were undertaken to test the hypothesis that Periplaneta americana (L.) are capable of adjusting their metabolic patterns to maintain homeostasis

with respect to energy and water balance. In all experiments in both studies, insects were housed in individual cages. The first study (Respirometric Study) consisted of 4 experiments designed to examine certain physiological responses under various conditions of food and water availability, as well as different relative humidities. In these experiments respiratory quotients (RQ), rates of oxygen consumption (QO_2), food and water consumption and insect weights were determined. Body weights, RQ and QO_2 were determined on days 0, 2, 4, 6, 8, 11, 13 and 15 in this study. Food and water consumption were determined on days 2, 4, 6, 8, 11, 13 and 15. The second study consisted of 6 experiments, in which radiolabelled substrates were used to determine the extent of radiolabel incorporation and metabolism in this insect under conditions of food and water availability, as well as exposure to 2 different relative humidities. In this study $^{14}CO_2$ was collected on days 1, 2, 4, 6, 8, 11, 13 and 15. Quantification of radiolabel incorporation into feces, fat body, hemolymph, cuticular and associated cuticular tissues and whole body was determined after 15 d.

Both the respirometric and radiolabel studies were conducted within enclosed experimental systems. These systems provided a temperature of 25 $^{o}C \pm 2\text{-}3\ ^{o}C$ and a 12L/12D photoperiod. The humidity and temperature were maintained and monitored with minimal disruption to the

encaged insects. To accomplish this, 2 different system designs were developed to maintain low (25-35% RH) and medium (55-65% RH) humidities in the respirometric study and low (25-35% RH) and moderate/high (70-80% RH) humidities in the radiolabel study.

Low humidities (25-35%) in both studies were maintained by passing air through 1500 g of Drierite and silica gel crystals prior to entering the enclosed systems. In the respirometric study, medium humidity was maintained by recirculating air through the 5.0 L plastic container (See Fig. 1). In the radiolabel study, air entering the moderate/high humidity chamber was first bubbled through 250 ml of distilled water.

To maintain temperature in both the studies, it was necessary to enclose individual housing units in constant temperature devices. In the respirometric study the 5.0 L plastic containers, containing the housing units, were placed in an environmental chamber. Temperature regulation in the radiolabel study is discussed in Section 3.2.1.

In the series of 4 respirometric experiments, conducted to determine if insect responses to experimental parameters were significantly different, insects were exposed to 2 different humidities (low = 25-35% RH and medium = 55-65% RH) and 4 different dietary regimens, over 15 d periods. The 4 dietary regimens were: Experiment 1 - insects were provided finely ground dog chow and distilled

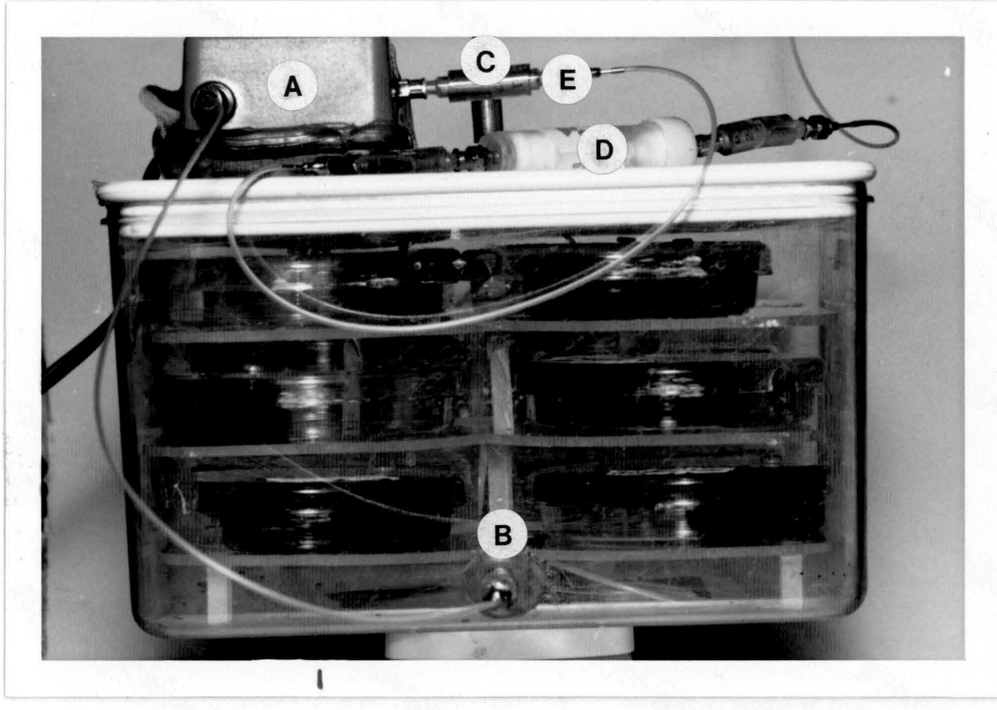


Figure 1. Medium humidity 5.0 L plastic container (respirometric study)

A = Aquarium pump equipped with incurrent and excurrent air ports
B = Container incurrent air connection
C = Container excurrent air connection
D = CO2 capture chamber containing KOH pellets
E = Incurrent air flow to aquarium pump

For specific details see Section 3.2

water ad libitum (FW); Experiment 2 - insects were provided only with distilled water ad libitum (OW); Experiment 3 - insects were provided only with finely ground dog chow ad libitum (FO); Experiment 4 - insects were denied both dog chow and water (OO). Food and water consumption and body weight changes were determined gravimetrically. Respiratory and oxygen quotients were determined by standard respirometric methods using a Gilson Differential Respirometer (Model # IGRP 4).

In the radiolabel study only 3 of the dietary regimens were used in both sets of experiments, since American cockroaches fed only dry food and no water do not feed (Tucker, 1977a). This response was also observed in the respirometric study. In Experiment I - insects were provided dog chow and distilled water ad libitum; Experiment II - insects were provided only with distilled water ad libitum; Experiment III - insects were deprived of both dog chow and water. As indicated above, 2 humidity regimens low (25-35% RH) and moderate/high (70-80% RH) were used in this study.

3.2.1 - INSECT HOUSING UNITS

In all experiments insects were individually housed in containers which provided relatively stable environmental

humidity and temperature which were monitored. However, these containers were different in the 2 studies. In the respirometric study, after humidity was recorded the insects were removed from their individual containers at various intervals and food and water consumption, body weight, RQ and QO_2 were determined. The insects in the radiolabel study were maintained in the individual housing units throughout the 15 d experimental period.

Insects used in the respirometric study were confined individually in modified, plastic petri dishes (20 mm X 85 mm) and provided with food and water as required by protocol (Fig. 2). Three tiers, each containing 4 housing units (3 experimental and 1 control), were stacked on one another (Fig. 3) and each humidity regimen was enclosed in a separate, sealed, 5.0 L, plastic container. The sealed containers were fitted with Luer-lok connections in order to allow for convenient, tight-sealing connections to tubing. Air was cycled through the enclosed 5.0 L plastic containers (at approximately 350-450 ml/minute) by means of aquarium pumps equipped with incurrent and excurrent air ports.

In the radiolabel study, insects were also individually housed, but in modified (40 mm X 60 mm) weighing dishes with ground glass seals. Pieces of 1/4 inch (in) Pyrex tubing were annealed to both the bottom and top portions to provide for incurrent and excurrent air

Figure 2. Modified plastic Petri dishes used as individual housing units (respirometric study)

A = Modified 1.5 ml, plastic microcentrifuge tube containing distilled water (respirometric study)
B = Suspended food container

For specific details see Section 3.2.1

Figure 3. Individual housing units stacked in tiers (respirometric study)

A = Individual housing unit
B = Plexiglass tier

For specific details see Section 3.2.1

ports. A 1/8 in mesh wire screen was suspended 3-5 mm from the bottom of the weighing dish. The weighing dishes were designed to accommodate feeding and watering devices when needed (Fig. 4). The modified weighing dishes were placed in 1 gal battery jars which were surrounded externally with 3/8 in Tygon tubing.

Six battery jar assemblies were connected in series by Tygon tubing wound around the battery jars and connected to a Lauda constant temperature water bath (Model # K-2/R). These 6 battery jars were separately housed in a large section of styrofoam (6.0 ft X 1.5 ft X 1.5 ft). Two units containing 6 battery jars were used in the study, one for each of the 2 humidities. The air (adjusted at the appropriate relative humidity) was pumped to the individual housing units via an incurrent manifold. Approximately 350-450 ml air/minute were passed simultaneously through all 12 of the housing units using a electric pump (Fisher Scientific - Model # 5KH33DN16). All connections from the pump to individual housing units, manifolds and pump returns were fabricated from modified 1 cc, plastic tuberculin syringe bodies equipped with Luer-lok fittings, 18 gauge, stainless-steel needles and Teflon tubing (1.14 mm I.D. and 1.57 mm O.D.) (Fig. 5).

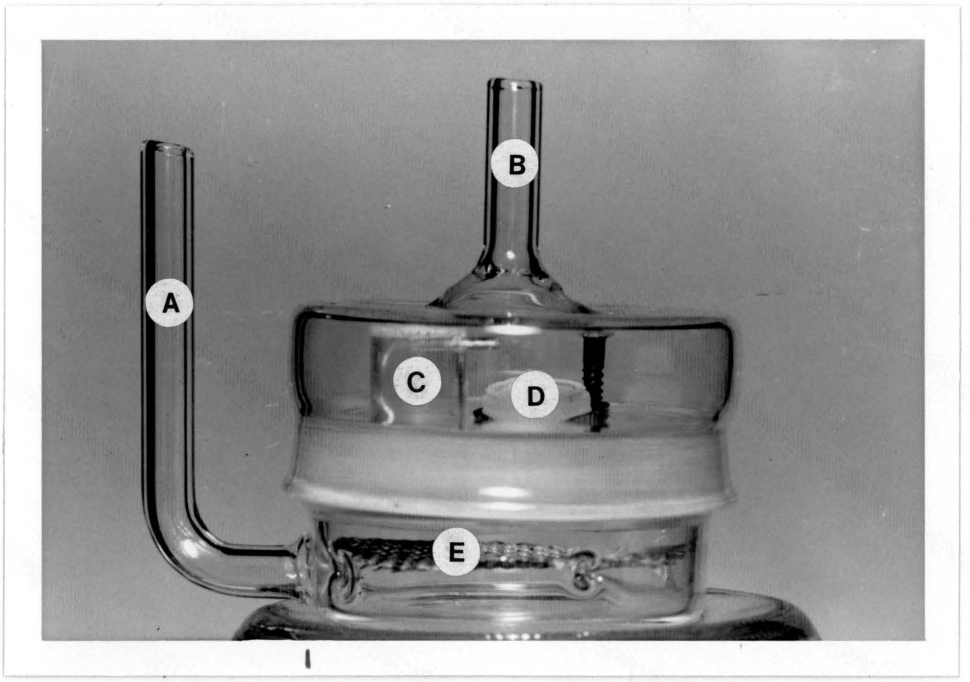


Figure 4. Modified weighing dish used as individual housing unit (radiolabel study)

A = Incurrent air port
B = Excurrent air port
C = Modified 1.5 ml, plastic sample vial containing distilled water
D = Suspended food container
E = 1/8 in mesh wire screen

For specific details see Section 3.2.1

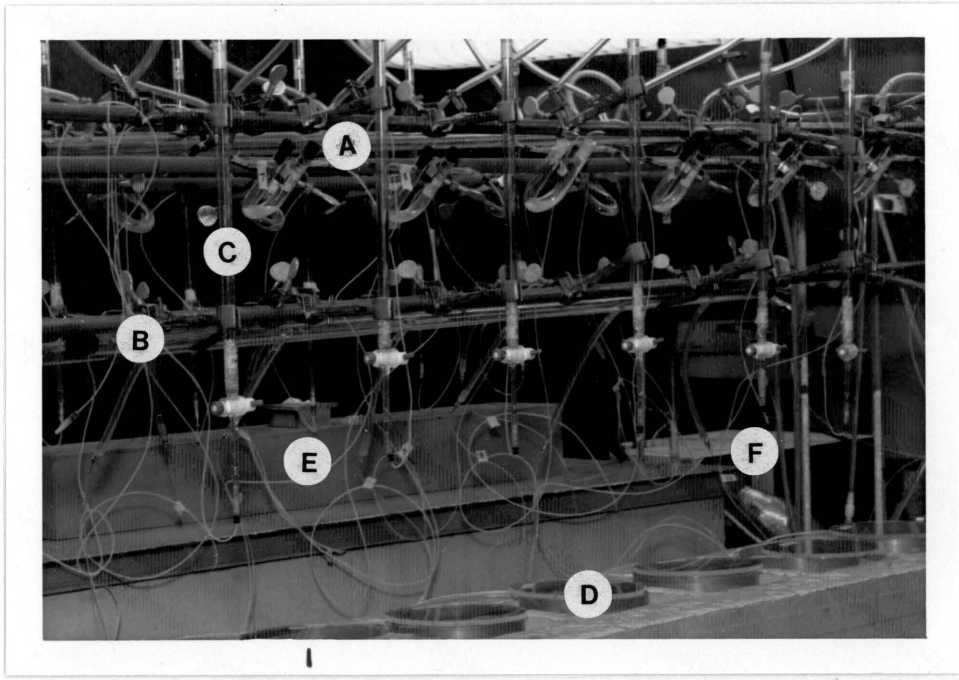


Figure 5. $^{14}CO_2$-trapping system containing individual housing units (radiolabel study)

A = Incurrent air manifold
B = Excurrent air manifold
C = 50 ml, CO_2 collection burrett
D = Battery jar containing individual housing unit
E = Lighting apparatus to maintain photoperiod
F = Lauda constant temperature water bath

For specific details see Sections 3.2.1 and 3.5

3.2.2 - HUMIDITY AND TEMPERATURE MONIOTRING

Humidity and temperature were monitored using a miniaturized psychrometer developed in our laboratory. Excurrent air from the enclosed systems passed through intramedic tubing (I.D. 1.14 mm and O.D. 1.57 mm) into a 1/4 in polyethylene "Y"-shaped connector. At this point the air was separated and passed, simultaneously, over two 0.125 mm Omega thermocouples (Chromel/Alumel). One thermocouple embedded in a knotted, water- soaked cotton wick was centered inside a 1.5 ml plastic, storage vial. This portion of the apparatus provided wet bulb temperature measurement of the air stream. A second thermocouple centered inside a dry 1.5 ml plastic, storage vial provided dry bulb temperature measurement. Air passed directly onto the thermocouples by means of modified 18 gauge, stainless-steel, hypodermic-needle tips centered inside the wet and dry bulbs. In this manner, both temperature and relative humidity of the circulating air stream could be determined simultaneously. All connections were fabricated from 1 cc tuberculin syringe bodies connected by Tygon tubing and fitted with 18 gauge, stainless- steel, hypodermic needles. Intramedic tubing was used between needle connections. This psychrometer was calibrated using a Honeywell Relative Humidity Sensor (Model #Q457) and an Atkins Pistol Psychrometer (Model #90023-F). Relative

humidity was calculated based on the difference between wet and dry bulb temperature determinations (Umbreit et al., 1964).

3.2.3 - DIET

In the respirometric study, food vials were removed from the individual housing containers and weighed immediately after they were dried to a constant weight by heating to 80-90 ^{o}C for 6 to 8 hr. The 24 h experimental food consumption values were determined on the basis of the average 15 d cumulative food consumption.

The Wayne dog chow diet contained (minimum) 25% crude protein, (minimum) 8% crude fat, (maximum) 4% crude fiber and (maximum) 12% moisture. This diet provided a well balanced assortment of metabolic substrates which an omnivorous insect, such as the American cockroach, might seek in its environment. The dog chow was finely ground to facilitate consumption and placed into small plastic vials which contained 800-1000 mg of diet. These plastic food vials were steamed for 30 minutes and oven dried at 80 ^{o}C overnight to solidify the food into cakes so the insects could not easily displace them while feeding.

Each food vial was suspended from the top of the individual housing units by means of a small wood screw positioned between a Tygon tubing washer (surrounding the

food vial) and the plastic food vial. In the respirometric study the wood screw was secured through the top of the plastic petri dish. In the radiolabel study, the wood screw was glued to the inside top of the modified glass weighing dish.

3.2.4 - WATER

In the respirometric study, water was provided in modified 1.5 ml, plastic, microcentrifuge tubes. A small hole was drilled in the top of each microcentrifuge tube large enough to allow a 1 mm (I.D.) glass tube with a cotton wick to be inserted. The cotton wick extended past the glass tube within the microcentrifuge tube and was slightly recessed externally to allow free wicking and less disturbance when the insects drank. The 1 mm glass tube portions of the water vials were placed through holes in the sides of the bottom portions of the Petri dishes, allowing free access by insects. The small water containers provided background evaporation rates (25 ul to 100 ul/24 hr) which allowed for increased accuracy in calculation of water consumption data obtained from individual insects (see Section 3.2.4.1).

Different water containers were employed in the radiolabel study. These were constructed from 1.5 ml,

plastic (cylindrical), sample vials with polyethylene caps. Water containers were glued to the inside top portions of the modified glass weighing dishes described in Section 3.3.1. A small piece of glass tubing (1 mm I.D.) was inserted through a hole near the glued portion of the water vial and a piece of water-saturated cotton string provided insects with a water source. The wick was recessed from the tip slightly to prevent its removal by the insect while drinking.

3.2.4.1 - WATER VIAL CALIBRATION

Water vials used in the respirometric study (Section 3.2.4) were calibrated in order to adjust for background evaporation rates. In some cases these rates were higher than some individual insect water consumption rates.

Water consumption was corrected for background evaporation by determining pre- and post-experimental evaporation rates for both experimental and a series of control water vials. A calibration value (adjustment factor due to evaporative water loss) was determined for each experimental water vial (based on the evaporative water loss in the control vial series) and used in the calculation of actual water consumption for the observational periods. The following 3 formulae were used to calibrate these vials and to estimate water loss due to

evaporation and that which was attributable to cockroach consumption.

1) $AWC_x = TWL_x - EWL_x$

Where:
AWC = actual water consumption
TWL = total water loss (observed)
EWL = estimated water loss (est. background evaporation)
x = a specific experimental vial

2) $EWL = \frac{C1+C2+C3}{3} \times CF_x$

Where:
C1 = Control vial 1
C2 = Control vial 2
C3 = Control vial 3
CF_x= Calibration factor for a specific vial (x), see equation 3 below

3) $$CF_x = \frac{\frac{PE1+PE2}{2}}{\frac{PC1+PC2}{2}}$$

Where:
CF_x = calibration factor for a specific vial (x)
PE1 = pre-experimental evaporation in experimental vial
PE2 = post-experimental evaporation in experimental vial
PC1 = pre-experimental evaporation average of 3 controls
PC2 = post-experimental evaporation average of 3 controls

If a water vial wick had been found to be dislodged by the insect (pulled partially out of the glass tube) at the time of water consumption determination, that vial's value was omitted from the calculations for that interval. Also, if a particular wick was out for more than 50% of the

observational intervals that vial was omitted from the overall experimental calculations. This procedure resulted in fewer experimental replicates (initial N = 9).

3.3 - RESPIROMETRIC METHODS

The insects were placed in modified respirometric chambers (open to the atmosphere) for equilibration (2 hr) prior to gasimetric analysis. Respirometric chambers used in this study consisted of modified 50 ml Erlenmeyer flasks (Fig. 6). The bottom portion of the 50 ml flask was annealed to the ground-glass top portion of a standard 15 ml Warburg flask. When necessary, the side arm contained KOH and paper wicks for CO_2 uptake.

After the equilibration period, 2-3 initial, 15-min, gas volume determinations were obtained, averaged and recorded. These initial observations were later used in the calculation of the insect's RQ for that interval. Next, 250 ul of 5.0 M KOH were added to the side arm of the flask to determine the O_2 consumption through measurement of CO_2 absorption. Following a second equilibration period of at least 1 hour, 2-3 consecutive, 15-min readings were obtained, averaged, recorded and used to calculate individual cockroach RQ and QO_2. The RQ's were determined using the following formula (Umbreit et al., 1964):

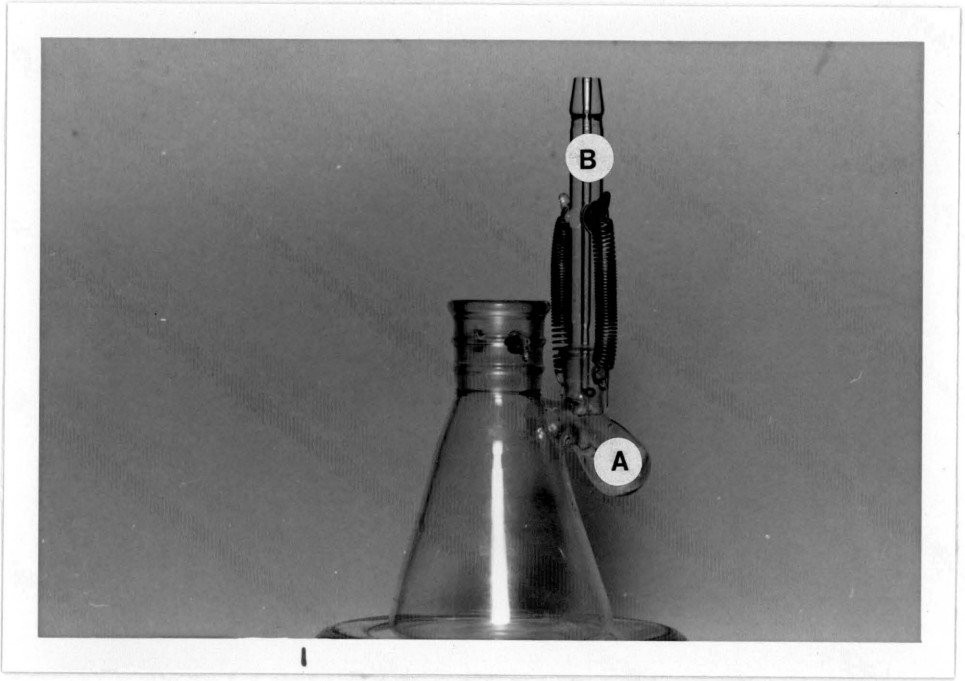


Figure 6. Modified 50 ml Erlenmeyer flask used as respirometric chamber (respirometric study)

A = Side arm containing fluted filter paper and distilled water
B = Respirometric chamber exhaust port

For specific details see Section 3.3

$$\text{Respiratory Quotient} = \frac{\text{ul } CO_2 \text{ Produced / Interval (hr)}}{\text{ul } O_2 \text{ Consumed / Interval (hr)}}$$

Where:

CO_2 Produced = gas volume determined with KOH Present - No KOH

O_2 Consumed = gas volume with KOH Present

An index useful in determining changes in metabolic rates, possibly indicative of stress, is the oxygen quotient or QO_2. This refers to a quantity of oxygen consumed/unit of body weight/unit of time. In these experiments it is expressed as ul O_2 consumed/mg body weight/hr.

3.4 - RADIOLABEL STUDY

To further examine the hypothesis regarding water/diet/humidity stress effects on substrate metabolism, 2 separate sets of radiolabelled substrate experiments (each consisting of 3 experiments) were conducted. The first set of 3 experiments involved the use of ^{14}C-glucose. In the second set of 3 experiments ^{14}C-palmitic acid was used. These radiolabelled substrates were injected ventrally between abdominal sternites 3 and 4. The

resulting radiolabel found as $^{14}CO_2$ (various intervals), whole body, feces, hemolymph, fat body, cuticle and associated cuticular material (all after 15 d) were determined and compared. Whole body assays of cockroaches injected at time 0 (controls) were done to obtain an estimated average total radiolabel injected into the experimental cockroaches. Radioactivity in the samples was determined using a Beckman Liquid Scintillation Counter (Model # LS-3150 T) and counted for 5 min at 0.7% error. Resulting background-corrected CPM were converted to DPM using a standard curve (obtained from a quenched ^{14}C standard series) and the External Standard Ratio (ESCR). Details on this procedure are outlined in the Beckman Liquid-Scintillation Counter Manual (1976, Beckman 3100 Series Liquid Scintillation Systems Instrument Manual, #015-082475).

3.4.1 - ^{14}C-GLUCOSE EXPERIMENTS

Five-hundred uCi of uniformly labelled ^{14}C-glucose (specific activity 9.1 mCi / mmole; lot #2671136) was obtained from ICN Biochemicals, Inc.. A stock solution containing 1.0 uCi/ul was prepared by dilution with 250 ul of water and 250 ul of 95% EtOH (500 ul total volume). This stock was used in all 3 of the ^{14}C- glucose

experiments (Section 3.2). Each insect was injected with 5.0 ul of a 0.29 uCi/ul solution (60 ul of stock solution + 150 ul distilled H_2O; total volume 210 ul). Therefore, each insect was injected with an estimated 1.45 uCi or approximately 3.2 X 10^6 (DPM).

3.4.2 - ^{14}C-PALMITIC ACID EXPERIMENTS

One-hundred uCi (2, 50 uCi ampules) of terminally labelled (C-16), ^{14}C-palmitic acid (specific activity 55.2 mCi / mmole; lot #44) was obtained from ICN Biochemicals, Inc. and used in this set of 3 experiments as described in Section 3.4. After evaporating benzene from each ampule (using nitrogen), a stock solution (of each ampule) containing 0.167 uCi/ul was prepared by dilution with 300 ul of technical grade acetone (300 ul total volume). Each cockroach was injected with 2.0 ul of the stock solution. Therefore, each cockroach (in all 3 experiments) was injected with an estimated 0.33 uCi or approximately 7.3 X 10^5 DPM.

3.5 - $^{14}CO_2$-TRAPPING SYSTEM

Respired $^{14}CO_2$ was collected from individual insects housed separately in modified weighing dishes (Section

3.2.1) by means of a CO_2-trapping system. Separate intake manifolds for each humidity regime contained 7 connections and the separate exhaust manifolds 6 connections. The seventh connection on each intake manifold was used to monitor the ambient environment. Therefore, air was partitioned into separate sides of the $^{14}CO_2$-trapping system (one side low humidity and one side moderate/high humidity), after being partitioned by a 1/4 in "Y"- shaped connector. The air then passed through the individual housing units and exited via separate exhaust ports. The air from each housing unit then passed through a 50 ml glass burette which contained 5.0 ml of 2.5 M KOH and 3.0 cm of 3.0 mm glass beads. Once $^{14}CO_2$ was removed from the air stream it passed to separate exhaust manifolds (1 for each humidity). The air from each exhaust manifold then passed through a 50 ml burette (1 on each side of the $^{14}CO_2$ trapping system = safety scrubber) containing 5-10 ml of 5.0 M KOH. The air from both safety scrubbers then was recombined (by another "Y"-shaped connector) and recirculated through the air pump to be recycled through the CO_2-trapping system. Quantification of the $^{14}CO_2$ from the KOH will be discussed below (Section 3.5.1).

3.5.1 - $^{14}CO_2$ QUANTIFICATION

Radiolabelled CO_2 trapped in the 5.0 ml of KOH (5.0 M) was removed from the individual insect and safety scrubbers and the glass beads rinsed 2 times with approximately 2.0 ml of distilled water each rinse. Air was then blown through the scrubbers (50 ml burettes) to remove most of the rinsate and the tip rinsed with approximately 1.0 ml of distilled water. The KOH and rinses were collected in 15 ml graduated centrifuge tubes and brought to 10 ml volumes with distilled water. A 3.0 ml aliquot was kept for analysis in 10 ml glass vials, with Teflon-lined caps.

The $^{14}CO_2$ assay methods were similar to those described by Mullins and Eaton (1977). Five hundred ul of the 3.0 ml KOH aliquot of scrubbing solution were placed into a 1.5 ml glass distillation well. The top of the distillation well was equipped with a hole which accommodated an 18 gauge, stainless-steel, hypodermic needle.

Prior to distillation, the interior of a 20 ml glass scintillation vial was completely coated with 500 ul of 80% ethanolamine. Each distillation well assembly was carefully placed into the ethanolamine-coated scintillation vial and the top screwed on lightly. Five hundred ul of 25 N H_2SO_4 (contained in a 1 cc tuberculin syringe) were delivered through the 18 gauge needle into the center of

the distillation well assembly. After the initial reaction of CO_2 release from the KOH/H_2SO_4 solution was achieved, the syringe was removed from the needle and the scintillation vial cap was screwed on firmly. The scintillation vials were placed in a tilted (45 ^{o}), rotating wheel and rotated at 6 revolutions per minute (RPM) for at least 2 hr. After the distillation process was completed, the scintillation vials were removed from the wheel and the reaction well, needle and modified cap removed along with the distillation well. Eight ml of scintillation cocktail (0.1 g, 1,4-bis-2-(5-phenyloxazolyl) benzene or POPOP, 4.0 g of 2,5-diphenyloxazol or PPO, 600 ml of toluene and 400 ml of 95% EtOH) were then added to the 500 ul of 80% ethanolamine containing the distilled $^{14}CO_2$.

3.6 - RADIOLABEL ASSAYS

Several fractions of the insects used in the radiolabel study were assayed for radiolabel content. Besides CO_2 (Section 3.5) feces, hemolymph, fat body, cuticle, associated cuticular materials and whole body assays were carried out to determine the fate of injected radiolabelled materials.

3.6.1 - FECES

Feces were removed from the individual housing units as completely as possible by careful scraping onto pretared weighing papers. The samples were weighed and placed in 1.5 ml polyethylene, microcentrifuge tubes. Five-hundred ul of distilled water was added to each tube and the feces homogenized by means of a miniature Teflon tissue homogenizer. The homogenates were centrifuged for 10 min at room temperature at medium speed (approximately 5-6000 RPM). A 100 ul aliquot of the supernatant was removed, placed in 8.0 ml of scintillation cocktail (xylene and PCS - Amersham grade, 1:1/V:V) and counted. This cocktail was used in all assays other than CO_2 determinations (refer to Section 3.5). Background-corrected CPM were calculated and adjusted to represent DPM/quantity of individual insect feces produced.

3.6.2 - HEMOLYMPH

Prior to hemolymph removal, insects were cooled for at least 1 hr along with 2.0 ul microcaps to facilitate hemolymph sampling. Hemolymph samples (0.5-2.0 ul) were usually obtained from clipped antennae, however, when necessary the metathoracic coxae were punctured in an attempt to obtain sufficient volumes. The whole hemolymph

collected was placed into 8.0 ml of xylene/PCS 1:1 scintillation cocktail and counted. Background-corrected CPM were calculated and adjusted to represent DPM/ul of whole hemolymph.

3.6.3 - CUTICLE

The pronotum was selected as a representative portion of the cockroach cuticle. All pronota were removed and the ventral side was scraped to remove epidermal tissue. Pronota were then weighed and placed in 1.0 ml of Beckman tissue solubilizer and incubated with agitation at 6 RPM overnight to clear samples of traces of adhering epidermal tissue.

Cleared pronota were rinsed in distilled water and each homogenized in 3.0 ml of cockroach saline at high speed (10) for 1 min with a Virtis tissue homogenizer (Model # "23"). A 500 ul aliquot was removed and placed in 8.0 ml of xylene/PCS 1:1 scintillation cocktail and counted. Background-corrected CPM were calculated and adjusted to represent DPM/pronotum.

Eight ml of xylene/PCS 1:1 scintillation cocktail were added to each ml of tissue solubilizer resulting from the incubation of the pronota. This solution was counted to determine the DPM incorporated into epidermal tissues and/or possible associated cuticular materials.

3.6.4 - FAT BODY

Fat body samples (including adhering tracheal tissue) were removed from the dorsal and lateral portions of the abdomen. Fat body tissue weights were recorded and 500 ul of cockroach saline were added to each sample. The mixture was homogenized (refer to Sec. 3.6.1), a 100 ul aliquot removed, placed in 8.0 ml of xylene/PCS 1:1 scintillation cocktail and counted after a 24 h period. Background-corrected CPM were calculated and adjusted to represent DPM/mg of wet fat body tissue.

3.6.5 - WHOLE BODY

Each cockroach carcass (minus pronota and fat body tissue samples) was cut into 25-30 small pieces and homogenized in 3.0 ml of cockroach saline at high speed (10) for 1 min using a Virtis tissue homogenizer (Model # "23"). A 500 ul aliquot was removed, placed in 8.0 ml of xylene/PCS 1:1 scintillation cocktail and counted after a 24 hr period. Background-corrected CPM were calculated and adjusted to represent DPM/cockroach carcass.

3.7 - STATISTICAL ANALYSIS

The number of individuals (N) used in each category (i.e. water consumption) of some of the experiments described differed due to mortality. Calculations and analysis of the data were adjusted for mortality or the inability to make observations at certain intervals.

The data from the respirometric, ^{14}C-glucose and ^{14}C-palmitic acid studies were analyzed using the Statistical Analysis Systems (SAS Institute, 1982). Initially, all data sets were checked for normality by analysis of the residuals. Sample sizes less than 51 are reported using the Shapiro-Wilk, W- statistic. Sample sizes greater than 51 are reported using the Dolomogorov, D-statistic. All data were subjected to Duncans Multiple Range Test (alpha = 0.05) and to ANOVA tests. Comparisons done on initial (0 d) and terminal values were done using the Standardized Students t-Test (alpha = 0.05).

4.0 - RESULTS/DISCUSSION

4.1 - RESPIROMETRIC STUDY

Preliminary analysis of the results obtained from the Respirometric Study indicated that the two humidity levels (low=25-35% RH and medium=55-65% RH) used in these experiments were not significant factors ($P < 0.05$) in producing significant differences in RQ, QO_2, food and water consumption and changes in body weight. Many of the statistical differences noted in the Respirometric Study were directly attributable to differences in dietary regimens. Therefore, the only independent variables used in the analysis were time interval (day) and dietary regimens (food and water ad libitum = FW, distilled water ad libitum = OW, dog chow ad libitum = FO and no dog chow or water = OO). Several tables provide detailed information associated with both humidity levels for comparative purposes. The dependent variables for the study were respiratory quotient (RQ), oxygen quotient (QO_2), food consumption (FC), water consumption (WC) and body weight (BW).

Information in Table 1 provides a summary of initial (0 d) and terminal (15 d) RQ values for all diets and both humidities. Information in this table requires some explanation in respect to initial and terminal RQ values. The initial RQ values recorded for low humidity and medium humidity insects likely reflect variation resulting from cyclic food and water consumption. Thus, initial values for these insects might have been more consistent if insects had been selected in the same phase of their dietary cycle. Cockroaches with varying initial quantities of energy reserves may react differently over periods of time in terms of utilization of energy reserves (Downer, 1983 and 1985).

Information provided in Table 1 indicates an initially low RQ value in medium humidity FW insects (0.74) and a significantly higher ($P < 0.05$) terminal RQ value (0.87). Low humidity control insects did not show a significant difference between initial and terminal RQ values. Therefore, the statistical significance observed between initial and terminal RQ values of the medium humidity control insects was probably unfounded. The OW insects in both humidities had initial (low humidity = 0.92 and medium humidity = 0.86) and terminal (low humidity = 0.71 and medium humidity = 0.68) RQ values which resulted in significantly different ($P < 0.05$) initial and terminal RQ values in this dietary regimen. The FO insects in both

TABLE 1 - Average initial and terminal RQ for adult, male American cockroaches maintained on 4 different dietary regimens and held under 2 different humidity levels for 15 d.

	LOW HUMIDITY[A] (25-35% RH)			
	FW	OW	FO	OO
I	(9) 0.83 ± 0.02	(9) $0.92_{A,a}$ ± 0.02	(9) 0.80 ± 0.03	(9) 0.79 ± 0.02
T	(8) 0.84 ± 0.02	(4) $0.71_{A,b}$ ± 0.04	(3) 0.87 ± 0.02	(3) 0.88 ± 0.09

	MEDIUM HUMIDITY[B] (55-65% RH)			
	FW	OW	FO	OO
I	(9) $0.74_{B,a}$ ± 0.01	(9) $0.86_{B,a}$ ± 0.03	(9) 0.82 ± 0.02	(9) $0.75_{B,a}$ ± 0.02
T	(9) $0.87_{B,b}$ ± 0.02	(8) $0.68_{B,b}$ ± 0.03	(6) 0.85 ± 0.03	(4) $0.87_{B,b}$ ± 0.03

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
FO = dog chow ad libitum (11 d)
OO = no dog chow or water (13 d)
I = initial (0 d) average respiratory quotient
T = terminal (15 d) average respiratory quotient
Table values = MEAN ± SEM
(N) = number of insects
Means (in columns within each humidity level) followed by the same upper and lower case letters are not significantly different (comparisons only between initial and terminal values for each treatment)
(Standardized Students t-Test; alpha = 0.05)

humidity levels had initial (low humidity = 0.80 and medium humidity = 0.82) and terminal (low humidity = 0.87 and medium humidity = 0.85) RQ values which were not significantly different. However, the medium humidity OO insects had an initially low RQ value (0.75) and a higher terminal RQ value (0.87) which resulted in a significant difference ($P < 0.05$) and possibly was unfounded. The low humidity OO insects did not show a significant difference in initial (0.79) and terminal (0.88) RQ values.

Since statistical significance was not clearly attributable to humidity differences in initial and terminal RQ, these values were combined to determine dietary regimen effect. The ANOVA on combined humidity RQ values, over the 15 d experimental period, indicated that significant differences in RQ values could be attributed to dietary regimens ($P < 0.0001$).

Information in Table 2 includes daily RQ data on insects on the 4 dietary regimens. Analysis indicated that after 2 d a significant rise (from 0.78 at 2 d to 0.85 at 4 d) occurred in RQ ($P < 0.05$) in the FW insects. The FW insects' RQ values fluctuated slightly after this initial rise, and their terminal RQ was significantly higher ($P < 0.05$) than that recorded at either 0 d or 2 d. The OW insects showed a significantly ($P < 0.05$) lower RQ after 2 d (0.89 at 0 d to 0.77 at 4 d). This reduction in RQ trend continued to 15 d (0.69 at 15 d). The FO insects exhibited

TABLE 2 - Average RQ values for adult, male American cockroaches maintained on 4 different dietary regimens (low and medium environmental humidities combined) for 15 d.

	RQ			
DAY	FW	OW	FO	OO
0	(17) 0.79[a]	(16) 0.89[a]	(18) 0.81[a]	(17) 0.77[a,b]
2	(17) 0.78[a]	(16) 0.86[a]	(18) 0.79[a]	(18) 0.80[a,b,c]
4	(17) 0.85[b]	(14) 0.77[b]	(18) 0.82[a]	(18) 0.79[a,b]
6	(16) 0.83[a,b]	(16) 0.75[b]	(18) 0.85[a]	(18) 0.75[a]
8	(17) 0.84[a,b]	(15) 0.75[b]	(17) 0.82[a]	(15) 0.81[a,b,c]
11	(17) 0.87[b]	(14) 0.68[b]	(9) 0.86[a]	(10) 0.85[b,c]
13	(15) 0.82[a,b]	(14) 0.71[b]	*(1) 1.00[b]	(3) 0.88[c]
15	(16) 0.86[b]	(12) 0.69[b]	--	--
OVERALL MEAN	0.83 ± 0.01	0.76 ± 0.03	0.83 ± 0.01	0.81 ± 0.02

RQ = average respiratory quotient (by day and dietary regimen)
FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
FO = dog chow ad libitum (11 d)
OO = no dog chow or water (13 d)
OVERALL MEAN = MEAN ± SEM
Table values = MEAN
(N) = number of insects
* = value not used in the AVG calculation because of low N
Means (within columns) followed by the same letter are not significantly different from each other (Duncans Multiple Range Test; alpha = 0.05)

a nonsignificant fluctuation in RQ from 0 d to 11 d (0.81 at 0 d to 0.86 at 11 d). Only 1 FO insect was alive at 13 d, and that insect had an RQ of 1.00. The OO insects showed an increase in RQ through 11 d (0.77 at 0 d to 0.85 at 11 d), and at 13 d their RQ (0.88) was the highest recorded at any point in their 13 d experimental period.

An analysis of average experimental RQ values within dietary regimens (excluding humidity) indicated that RQ values for FW (0.83 over 15 d), FO (0.83 over 11 d - last day considered statistically) and OO (0.81 over 13 d) insects were similar, and that all 3 were significantly higher than OW (0.76 over 15 d) insects ($P < 0.05$). In addition, OW (0.69 at 15 d) and OO (0.88 at 13 d) insect terminal RQ values were significantly different from each other ($P < 0.05$).

Based on the results presented in Table 2 it appears that OW insects change their initial metabolic substrate utilization pattern over the 15 d experimental period. These insects had an initial RQ value of 0.89 and a 15 d terminal RQ value of 0.69, indicative of lipid-based substrate metabolism.

Since the FW, FO and OO insects showed similar terminal RQ values (FW = 0.86 at 15 d, FO = 0.86 at 11 d and OO = 0.88 at 13 d) it is assumed that either there was no difference in the substrate being metabolized (based only on RQ values) or that the substrates (possibly

different) yielded approximately the same RQ value upon combustion. These findings disagree with those of Tucker (1977c) in which she reported that a few dehydrating (no food or water) adult, male American cockroaches had an average RQ value of 0.76, indicating lipid-based substrate metabolism. RQ data in these experiments do not indicate that dehydrating cockroaches change their initial metabolic substrate utilization pattern, over the experimental period, to a lower (ie. primarily lipid-based) metabolism, but appear to maintain a constant or slightly elevated RQ value.

4.1.2 - OXYGEN QUOTIENTS (QO_2)

QO_2, an index of basal metabolism pattern change (other than RQ), is related to metabolic activities and reflects rate changes in oxygen consumption. An attempt was made to correlate QO_2 values with changes in RQ values in order to compare changes in oxygen utilization rates recorded in dietary regimens with changes in primary metabolic substrate utilization patterns. The trends in QO_2 for the Respirometric Study are discussed below.

The information in Table 3 summarizes initial (0 d) and terminal QO_2 values obtained from adult, male American cockroaches on all 4 diets and under 2 humidity levels.

TABLE 3 - Average initial and terminal QO_2 values for adult, male American cockroaches maintained on 4 different dietary conditions and held under 2 different humidity levels for 15 d.

	LOW HUMIDITY (25-35% RH)			
	FW	OW	FO	OO
QO_2I	(9) 0.38 ± 0.04	(9) 0.45 ± 0.03a	(9) 0.34 ± 0.03	(9) 0.37 ± 0.02a
QO_2T	(8) 0.38 ± 0.01	(4) 0.28 ± 0.03b	(3) 0.43 ± 0.03	(3) 0.53 ± 0.03b
	MEDIUM HUMIDITY (55-65% RH)			
QO_2I	(9) 0.37 ± 0.02	(9) 0.35 ± 0.02	(9) 0.45 ± 0.02	(9) 0.32 ± 0.01a
QO_2T	(9) 0.35 ± 0.01	(8) 0.30 ± 0.03	(6) 0.49 ± 0.05	(4) 0.45 ± 0.04b

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
FO = dog chow ad libitum (11 d)
OO = no dog chow or water (13 d)
QO_2I = average 0 d oxygen quotient (ul O_2/mg body weight/h)
QO_2T = average 15 d oxygen quotient
Table values = (N) MEAN ± SEM
(N) = number of insects
Means (within columns within humidity levels) followed by the same letter are not significantly different from each other (Standardized Students t-Test, alpha = 0.05)

Information presented in this table suggests that FW low and medium humidities had initial and terminal QO_2 values which were very similar (low humidity - both 0 d and 15 d = 0.38 and medium humidity 0 d = 0.37 and terminal, 15 d = 0.35), suggesting little, if any, change. In low humidity OW insects there was a significant difference ($P < 0.05$) in QO_2 when 0 d (0.45) was compared to 15 d (0.28). A significant decrease in QO_2 (initial QO_2 compared to terminal QO_2) was not observed in any of the other dietary regimens over respective experimental periods. Information in Table 3 also indicates that a significant ($P < 0.05$) rise from initial to terminal QO_2 occurred in both low and medium humidity OO insects (low humidity 0 d = 0.37 and terminal, 13 d = 0.53; medium humidity 0 d = 0.32 and terminal, 13 d = 0.45). This trend was also observed over the experimental period in both low and medium humidity FO insects (low humidity 0 d = 0.34 and terminal, 11 d = 0.43; medium humidity 0 d = 0.45 and terminal, 11 d = 0.49). Even though this rise in QO_2 in FO insects was not found to be significant, terminal QO_2 values in both humidities were higher in this dietary regimen than those terminal values recorded for both the FW (low humidity = 0.38 and medium humidity = 0.35) and OW (low humidity = 0.28 and medium humidity = 0.30) insects.

Information in Table 4 summarizes the average QO_2 values obtained from adult, male American cockroaches in 4 different dietary regimens (humidity levels combined) over respective experimental periods. In the FW insects, QO_2 values fluctuated from 0 d through 15 d, and both 0 d (0.38) and 15 d (0.36) were not significantly different from each other. The OW insects exhibited a QO_2 trend different than that of FW insects. Their 0 d QO_2 value (0.40) was higher than those recorded on any other day of the experiment. After several fluctuations, their 15 d QO_2 value (0.30) was significantly lower ($P < 0.05$) than the 0 d value. No logical explanation can be provided at this time for these results, as one owuld expect oxygen consumption rates to be higher in insects metabolizing primarily lipids. The FO insects QO_2 values decreased from 0 d (0.39) through 4 d (0.34), followed by a rise recorded at 6 d (0.43) slightly above initial values. QO_2 values recorded at 8d and 11 d (0.50 and 0.47, respectively) were the highest values recorded throughout the experimental period for this group, but were not found to be significantly different from 0 d values. The lowest QO_2 value was recorded at 13 d (0.31), although only one insect was alive. The clearest QO_2 trend occurred in the OO insects. Here insects showed a significant rise ($P < 0.05$) in QO_2 from 4 d-13 d [0.34 at 4 d to 0.53 at 13 d (last

TABLE 4 - Average QO_2 values obtained from adult, male American cockroaches maintained on 4 different dietary regimens (low and medium environmental humidities combined) for 15 d.

	QO_2			
DAY	FW	OW	FO	OO
0	(18) $0.38^{a,b}$	(18) 0.40^{a}	(18) $0.39^{a,b}$	(18) 0.35^{a}
2	(18) $0.38^{a,b}$	(18) $0.36^{a,b}$	(18) 0.34^{b}	(18) 0.33^{a}
4	(18) 0.40^{a}	(17) 0.30^{b}	(17) 0.34^{b}	(18) 0.34^{a}
6	(18) 0.41^{a}	(16) $0.35^{a,b}$	(18) $0.43^{a,b}$	(18) 0.48^{b}
8	(18) $0.36^{a,b}$	(15) 0.31^{b}	(17) 0.50^{a}	(15) 0.47^{b}
11	(18) 0.40^{a}	(14) 0.30^{b}	(9) 0.47^{a}	(10) 0.48^{b}
13	(18) 0.33^{b}	(15) $0.33^{a,b}$	*(1) 0.31^{b}	(4) 0.53^{b}
15	(17) $0.36^{a,b}$	(12) 0.30^{b}	--	--
OVERALL MEAN	0.38 ± 0.01^{a}	0.33 ± 0.01^{b}	0.41 ± 0.03^{c}	0.43 ± 0.03^{c}

QO_2 = average oxygen quotient (ul/mg/hr)
FW^2 = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
FO = dog chow ad libitum (11 d)
OO = no dog chow or distilled water (13 d)
OVERALL MEAN = MEAN $\pm$ SEM
Table values = MEAN
(N) = number of insects
* = value not used in AVG calculation because of low N
Means (within columns) followed by the same letter are not significantly different from each other (Duncans Multiple Range Test; alpha = 0.05)

experimental day of the OO insects)]. The highest recorded QO_2 (0.53) of any dietary regimen was recorded at 13 d in the OO insects.

The overall experimental average FO (0.41) and OO (0.43) QO_2 values were both significantly higher than those of FW (0.38) and OW (0.33) insects ($P < 0.05$). Also, the FW and OW insects were significantly different ($P < 0.05$) from each other. Results indicate that of the dietary regimens examined with respect to terminal QO_2 values, the OO insects showed the highest QO_2 value (0.53) and OW insects the lowest QO_2 value (0.30). The fluctuations in QO_2 in FW insects may represent a cyclic oxygen utilization pattern associated with cyclic feeding and drinking characteristics of American cockroaches. Banks, et al. (1975) reported adult Blaberus craniifer males and P. americana had similar oxygen consumption rates between 16°C and 26°C of approximately 0.17 ul/mg/hr. Insects in this study provided food and water ad libitum and held at 25°C, had oxygen consumption rates of 0.38 ul/mg/hr (refer to Table 4). This value represents an experimental average over a 15 d period, whereas insects examined by Banks et al. were studied over 24 hr periods, and insects were deprived of food and provided water prior to their initiation into the experiment.

4.1.3 - FOOD CONSUMPTION

Information in Table 5 provides information on 15 d average food consumption in adult, male American cockroaches maintained on 2 (FW and FO) dietary regimens and held under 2 humidity levels in the Respirometric Study. Food consumption between FW and FO insects was significantly different ($P < 0.0001$) over respective experimental periods (FW low humidity = 20.6 mg/d and medium humidity = 29.4 mg/d; both low and medium humidity FO = 0.7 mg/d).

The FW insects (evaluation of combined humidities) showed a rise in average food consumption from 2 d (7.6 mg - lowest average food consumption observational period) through 11 d (40.5 mg - highest average food consumption). From 11 d-15 d, a noticeable decline occurred (15 d avg. = 28.1 mg). These insects consumed significantly less food ($P < 0.05$) 0 d-2 d and significantly more food ($P < 0.05$) at 11 d than at any of the other days recorded. Hyatt and Marshall (1985) reported an average daily food consumption (over an 8 d period with dog chow) for adult, male American cockroaches of 44.6 ± 11.5 mg/d. It appears that confined cockroaches provided with both food and water, for periods of greater than 8 d, may reduce their average food consumption.

TABLE 5 - Average food consumption (mg) in adult, male American cockroaches maintained on FW and FO dietary regimens and held under 2 different humidity levels for 15 d.

	FO		FW	
DAY	LOW	MED	LOW	MED
2	(9) 1.9	(9) 0.2	(9) 3.1	(8) 12.0
4	(9) -0.1	(9) 0.8	(9) 13.7	(8) 26.9
6	(9) 0.9	(9) 0.8	(9) 12.6	(8) 26.3
8	(9) -2.0	(9) 1.6	(9) 21.2	(8) 39.0
11	(9) 3.0	(9) -0.1	(9) 42.9	(8) 38.1
13	--	--	(9) 24.4	(8) 33.3
15	--	--	(9) 26.2	(8) 30.0
OVERALL MEAN	0.7 ± 0.9^a	0.7 ± 0.3^a	20.6 ± 4.8^b	29.4 ± 3.5^b

FO = dog chow ad libitum, no water (11 d)
FW = dog chow and distilled water ad libitum (15 d)
LOW = low humidity (25-35% RH)
MED = medium humidity (55-65% RH)
OVERALL MEAN = sum of average food consumption for observations/ number observations (values = MEAN ± SEM)
Table values = MEAN
(N) = number of insects
Means followed by the same letter are not significantly different from each other (Duncans Multiple Range Test; alpha - 0.05)

The highest average food consumption value for the FO insects was recorded at 11 d (1.5 mg) and their lowest average value at 8 d (-0.2 mg). Low humidity FO insect 8 d food value of -2.0 mg probably reflects some type of error measurement when the food vial was weighed. The observation that significantly less ($P < 0.0001$) average food consumption in FO insects (Table 5) supports the findings made by other workers (Wall, 1970 and Tucker, 1977a), that allowed only dry food and no water, cockroaches eat very little, if any.

4.1.4 - WATER CONSUMPTION

Information in Table 6 presents 15 d average water consumption obtained for FW and OW insects under both humidity levels during the Respirometric Study. No significant differences in average water consumption were observed in respect to dietary or humidity regimens. The mean drinking rates in this table suggest that low humidity FW insects (44.3 ul/d) drank less water than medium humidity FW insects (69.9 ul/d), but that low humidity OW insects (76.6 ul/d) drank more than medium humidity OW insects (45.0 ul/d). The low humidity FW insect drinking value seems low compared to that of the medium humidity insects considering low humidity insects should have higher

TABLE 6 - Average water consumption (ul) in adult, male American cockroaches maintained on FW and OW dietary regimens[1] and held under 2 different humidity levels for 15 d.

	LOW HUMIDITY (25-35% RH)		MEDIUM HUMIDITY (55-65% RH)	
DAY	FW	OW	FW	OW
2	(9) 31.0	(6) 29.2	(8) 46.9	(8) 17.5
4	(8) 19.7	(6) 98.7	(8) 37.7	(8) 46.6
6	(9) 40.4	(6) 31.8	(8) 81.7	(8) 44.6
8	(8) 50.2	(5) 60.2	(8) 79.8	(8) 73.9
11	(6) 19.3	(5) 109.2	(8) 80.7	(7) 49.1
13	(8) 54.0	(5) 107.4	(8) 88.6	(7) 20.9
15	(8) 95.2	(4) 100.0	(7) 74.2	(6) 62.3
OVERALL MEAN	44.3 ± 9.9	76.6 ± 13.4	69.9 ± 7.4	45.0 ± 7.7

[1]No significant differences were found when tested using Duncans Multiple Range Test (alpha = 0.05)
FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OVERALL MEAN = sum of average mean water consumptions for observations/number observations (values = MEAN ± SEM)
Table values = MEAN
(N) = number of insects

water demands than those of medium humidity insects. Similar results under comparable conditions were observed in some preliminary studies in this lab. Heit et al. (1973) reported that adult, male American cockroaches (fed dog chow) had a 7 d average water consumption of 21 ul/d. The water vial and calculation of background water evaporation used in calibration (Section 3.3.4.1) may be more reliable than some types used in earlier studies, therefore may reflect different water consumption values than previously reported. Also, Tucker (1977a) reported that confinement of American cockroaches held under humidities as low as 25% RH resulted in decreased physical activity, which could result in decreased feeding and drinking. In addition, Hyatt and Marshall (1985) reported adult, male American cockroaches drink approximately every 3 d, and confinement in the later portion of the 3 d cycle may result in the mobilization of antidiuretic factors and fat body tissues. This suggests increased metabolism of lipid substrates in an attempt to supplement reduced water availability.

ANOVA indicated that of FW and OW insects (humidities combined) only FW insects exhibited a significant difference in average water consumption ($P < 0.0180$) over the 15 d experimental period. The FW insects (humidities combined) had their highest average water consumption recorded at 15 d (84.7 ul) and their lowest average

consumption at 4 d (28.7 ul). The OW insects had their highest average water consumption recorded at 15 d (81.2 ul) and their lowest at 2 d (23.4 ul).

4.1.5 - BODY WEIGHT

Information in Table 7 summarizes average body weight information obtained from adult, male American cockroaches maintained on 4 different dietary regimens and held under 2 different humidities. Statistical analysis indicated that there were significant differences ($P < 0.05$) in average terminal body weight among dietary regimens. The FW insects had the highest average body weight (896 mg: low humidity = 813 mg and medium humidity = 980 mg) followed by OW insects (821 mg: low humidity = 843 mg and medium humidity = 799 mg), OO insects (701 mg: low humidity = 684 mg and medium humidity = 717 mg) and FO insects (668 mg: low humidity = 683 mg and medium humidity = 652 mg).

Within the dietary regimens examined, only FO and OO insects showed significantly lower ($P < 0.0001$) average terminal body weights at 15 d. FW and OW insects showed no significant changes in respect to initial and terminal body weight over the 15 d experimental period. This is consistent with what might be expected and what is reported in the literature. On the other hand, American cockroaches

TABLE 7 - Average body weight (mg) in adult, male American cockroaches maintained on 4 different dietary regimens and held under 2 different humidity levels for 15 d.

LOW HUMIDITY (25-35% RH)

DAY	FW	OW	FO	OO
0	(9) 815	(8) 887	(9) 815	(9) 846
2	(9) 788	(8) 842	(9) 752	(9) 759
4	(9) 766	(8) 872	(9) 699	(9) 703
6	(9) 766	(8) 799	(9) 643	(9) 656
8	(9) 808	(7) 826	(9) 586	(8) 627
11	(9) 861	(7) 827	(3) 605	(6) 615
13	(9) 850	(7) 830	--	(3) 579
15	(8) 849	(5) 857	--	*(1) 502
MEAN	813 ± 13	843 ± 10	683 ± 36	684 ± 35

MED HUMIDITY (55-65% RH)

DAY	FW	OW	FO	OO
0	(8) 832	(8) 846	(9) 761	(9) 856
2	(8) 828	(8) 794	(9) 712	(9) 778
4	(8) 854	(8) 801	(9) 666	(9) 738
6	(8) 872	(8) 777	(9) 625	(9) 686
8	(8) 902	(8) 805	(8) 592	(9) 631
11	(8) 899	(8) 802	(6) 554	(6) 616
13	(8) 919	(8) 769	*(1) 578	*(1) 570
15	(8) 933	(8) 800	--	--
MEAN	980 ± 14	799 ± 8	652 ± 31	717 ± 37
OVERALL MEAN	896 ± 84[a]	821 ± 22[b]	668 ± 16[c]	701 ± 17[d]

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
FO = dog chow ad libitum (11 d)
OO = no dog chow or water (13 d)
MEAN = sum of average body weights from observations/number of observations (values = MEAN ± SEM)
OVERALL MEAN = MEAN ± SEM (low and medium humidity values used)
Table values = MEAN
(N) = number of insects
Means followed by the same letter are not significantly different from each other (Duncans Multiple Range Test; alpha = 0.05)
* = value not used in the AVG calculation because of low N

allowed no water to drink for extended periods lose significant body weight (Wall, 1970; Hyatt and Marshall, 1977; Tucker, 1977a). Wall (1970) found that adult, male cockroaches when dehydrated lost an average of 10-12 mg/insect/d. Hyatt and Marshall (1977) reported that adult, male cockroaches held under 60% RH lost 50% of their body weight after 8 d of dehydration. Tucker (1977a) reported a 30% decrease in body weight for adult, male cockroaches dehydrated for 12 d at humidities as low as 25% RH.

Information in Table 8 summarizes average body weight changes in adult, male American cockroaches maintained on 4 different dietary regimens and under 2 different humidity levels in the Respirometric Study. The FO and OO insects in both humidities were found to contain percent water values (FO low humidity = 62.6% and OO low humidity = 58.9%; FO medium humidity = 62.8% and OO medium humidity = 62.7%) similar to those of adult, male American cockroaches reported by Tucker (1977a) dehydrated for 13 d (56%). The results in Table 8, with respect to OW insects only, agree with Wharton et al. (1965) in that starvation increases the percent water content of the cockroach in relation to its weight. Higher dry weight percentages of FO and OO insects reflect severe body water loss, as expected.

TABLE 8 - Average body weight change information for adult, male American cockroaches maintained on 4 different diets and held under 2 different humidity levels.

LOW HUMIDITY (25-35% RH)

	FW	OW	FO	OO
IBW	(9) 815 ± 35	(8) 887 ± 41	(9) 815 ± 28	(9) 846 ± 21
TBW	(8) 849 ± 37	(5) 857 ± 30	(3) 605 ± 45	(3) 579 ± 19
DW	(9)265 ± 12	(9) 238 ± 11	(9) 226 ± 9	(9) 238 ± 9
% BWC	(9) +4.2	(9) -3.4	(9) -25.8	(9) -31.6
% WATER	68.8	72.2	62.6	58.9
% DRY WT	31.2	27.8	37.4	41.1

MEDIUM HUMIDITY (55-65% RH)

	FW	OW	FO	OO
IBW	(8) 832 ± 26	(8) 846 ± 36	(9) 761 ± 29	(9) 856 ± 22
TBW	(8) 933 ± 37	(8) 800 ± 43	(6) 554 ± 34	(6) 616 ± 12
DW	(9) 273 ± 13	(9) 220 ± 12	(9) 206 ± 9	(9) 236 ± 9
% BWC	+12.1	-5.4	-27.2	-28.0
% WATER	70.7	72.5	62.8	62.7
% DRY WT	29.3	27.5	37.2	37.3

FW = dog chow and distilled water ad libitum (≤ 15 d)
OW = distilled water ad libitum (≤ 15 d)
FO = dog chow ad libitum (≤ 11 d)
OO = no dog chow or water (≤ 13 d)
IBW = average initial wet body weight (0 d)
TBW = average terminal wet body weight (15 d)
DW = average dry weight
% BWC = TBW/IBW X 100
% WATER = 100% - %DW (value assumed to be % water)
% DRY WT = DW/TBW X 100
Table values = other than % are MEAN ± SEM
(N) = number of insects

4.2 - RADIOLABEL STUDY

The Radiolabel Study was comprised of 2 sets of experiments, the ^{14}C-glucose experiments and the ^{14}C-palmitic acid experiments (Section 3.5). Both experiments were conducted to determine the quantity of radiolabel incorporation into whole body, feces, hemolymph, fat body and cuticle and associated cuticular material at 15 d, after exposure to 3 different dietary regimens and 2 levels of relative humidity. Cumulative radiolabel released as $^{14}CO_2$ is reported over respective experimental periods.

4.2.1 - ^{14}C-GLUCOSE EXPERIMENTS

Preliminary analysis of the 3 radiolabel glucose experiments indicated that, as in the Respirometric Study (Section 4.1), humidity generally was not a major factor in producing significantly different results with respect to radiolabel incorporation into whole body, hemolymph and cuticle and associated cuticular materials. However, analysis of feces and fat body tissue did indicate that significant differences in radiolabel incorporation appeared to be related to the 2 humidity levels (low=25-35% and moderate/high=70-80% RH) in which the cockroaches were maintained. Because of this, most tables will include

information obtained from both humidity levels for comparative purposes. It should be noted that none of the low humidity OO insects survived to 15 d. This situation affects ultimate interpretation of the results in the following sections.

4.2.2 - OVERVIEW

Information in Table 9 summarizes radiolabel in whole body, feces and CO_2 compared to both the average zero whole body (control insects) content recorded for each dietary regimen after injection with radiolabelled glucose and theoretical injected amounts. Although the differences were not found to be significant, information presented in this table implies that OW insects may have retained more radiolabel in their bodies at 15 days (401,730 DPM) than either FW insects (307,074 DPM) or OO insects (326,896 DPM). Also, OW insects appeared to release more radiolabel in the form of $^{14}CO_2$ (2,036,356 DPM) than either FW or OO insects (1,826,181 DPM and 593,923 DPM, respectively). In addition, the OO insects appeared to incorporate more fecal radiolabel (13,845 DPM) than either FW or OW insects (12,489 DPM and 9,339 DPM, respectively).

The low radiolabel recovery from the zero time (injected controls) insects (FW = 76.5%, OW = 91.8% and OO

TABLE 9 - Radiolabel incorporation in whole body, feces and CO_2 as compared to average zero time insects in adult, male American cockroaches maintained on 3 different dietary regimens 15 d after injection with ^{14}C-glucose.

	DPM		
	FW	OW	OO
T(0)	(4) 1,731,199 ± 117,777	(4) 1,922,522 ± 115,247	(4) 2,443,463 ± 121,682
T(IC)	(6) 2,262,766 ± 3,285	(8) 2,093,969 ± 9,557	(8) 2,940,389 ± 54,502
% REC	76.5	91.8	83.1
WB	(9) 307,074 ± 30,214	(10) 401,730 ± 58,681	(6) 326,896 ± 53,065
FECES	(9) 12,489 ± 2,044	(10) 9,339 ± 1,533	(6) 13,845 ± 2,068
CO_2	(9) 1,826,181 ± 177,245	(11) 2,036,356 ± 5,874	(6) 593,923 ± 79,522
TOTAL	2,145,744	2,447,425	934,664
% T(0)	124	127	38
% T(IC)	95	117	32

T(0) = average DPM recovered from zero time injected control insects/avg. body weight
T(IC) = average DPM/(N) applications to filter paper strips before, during and after injections of radiolabelled glucose
% REC = T(O)/T(IC) X 100
FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
OO = no dog chow or water
WB = average DPM incorporation/avg. wet body weight (15 d)
FECES = average DPM incorporation/avg. total fecal production (15 d)
CO_2 = average total DPM in $^{14}CO_2$ (15 d)
TOTAL = WB + FECES + CO_2
% TOTAL (0) = TOTAL/TOTAL (0)
% TOTAL (IC) = TOTAL/TOTAL (IC)
Table values = MEAN ± SEM
(N) = number of insects

= 83.1%), compared to theoretical injected amounts [T(IC)] may explain the large % T(O) for FW and OW insects (124% and 127%, respectively). The recovery (38%) of radiolabel from OO insects 15 d whole body, feces and CO_2 is low when compared to zero time (injected controls) whole body radiolabel incorporation. This possibly is the result of radiolabel incorporation into fractions not assayed (ie. methane production) or failure to account for residues which may have remained in the individual housing units after fecal collection was carried out. Specific information on whole body, feces and CO_2 are reported below. When radiolabel incorporation into 15 d whole body, feces and CO_2 are compared to the T(IC) FW insects show 95% recovery, OW insects 117% and OO insects 32%. No satisfactory explanation of these extremely low OO insect recoveries [38% (% T(O) and 32% (% T(IC)] can be made without further investigation.

4.2.2.1 - WHOLE BODY

Information presented in Table 10 summarizes the 15 d average whole body weights, radiolabel/insect, and average DPM/zero time whole body and average estimated total injection in adult, male American cockroaches maintained on 3 different dietary regimens

TABLE 10 - Whole body weights, total radiolabel/insect and radiolabel/zero time insect in adult, male American cockroaches maintained on 3 different diets 15 d after injection with ^{14}C-glucose.[1]

	FW	OW	OO
BW	(9) 807.2 ± 50.5	(11) 789.0 ± 39.7	(6) 705.0 ± 37.0
TOTAL DPM/INSECT	(9) 307,074 ± 30,214	(10) 401,730 ± 58,681	(6) 326,896 ± 53,065
DPM INJ (CONTROL)	1,731,199 ± 117,777	1,922,522 ± 115,247	2,443,463 ± 121,682
INSECT %	17.7%	20.8%	13.4%
IC	(6) 2,262,766 ± 3,285	(8) 2,093,969 ± 9,557	(8) 2,940,389 ± 54,502

[1]No significant differences were found when tested using Duncans Multiple Range Test (alpha = 0.05)
FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
OO = no dog chow or water
BW = average wet body weight (15 d)
TOTAL DPM/INSECT = average DPM incorporation/average wet body weight (15 d)
DPM INJ (CONTROL) = average DPM recovered from zero time insects/average body weight (N = 4)
INSECT % = (TOTAL DPM/INSECT)/DPM INJ (CONTROL)
IC = average DPM/(N) applications to filter paper strips before, during and after insect injections of radiolabelled glucose
Table values = MEAN ± SEM
(N) = number of insects

and injected with radiolabelled glucose. Whole body analysis at 15 d indicated no significant differences with respect to incorporation of radiolabel/insect due to dietary regimens. Comparing the 15 d total insect DPM to average zero time DPM, FW insects retained 17.7% of the radiolabel, while the OW insects retained 20.8% and OO insects 13.4%. Although no significant differences were found, the results suggest that cockroaches deprived of food, but provided with water (OW insects), may rely on reserves other than carbohydrate, since they retained a slightly higher percent of radiolabel originating from glucose than controls (FW insects). Also, OW insect data agree with those of Cervenkova (1960) and Downer (1983 and 1985), in that starved cockroaches appear to utilize non-carbohydrate reserves (possibly lipids in this case) more heavily when stressed. The OO insects (retaining less radiolabel, 13.4%) suggest that the injected glucose was used more heavily or at a higher rate (RQ and QO_2 data support this suggestion). This information tends to reject the hypothesis that dietarily stressed OO insects would metabolize primarily lipids, after carbohydrate stores are depleted.

4.2.2.2 - FECES

Information presented in Table 11 summarizes 15 d total radiolabel fecal production (mg) in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels. The results indicate that moderate/high humidity FW insects produced significantly more ($P < 0.05$) fecal material than low humidity FW insects (95.3 mg and 26.0 mg, respectively). Also, low humidity FW and OW insects incorporated significantly more ($P < 0.05$) radiolabel in their feces (19,889 DPM and 12,824 DPM, respectively) than corresponding moderate/high humidity FW and OW insects (8,791 DPM and 7,461 DPM, respectively). The moderate/high humidity OO insect radiolabel fecal value (17,136 DPM) cannot be compared to a corresponding low humidity OO insect value, as none of the low humidity insects survived to 15 d. The moderate/high humidity OO insect value was similar to both low humidity FW and OW insects suggesting that food and water deprivation and low humidity conditions may have similar effects in this instance. Therefore, conclusive evaluation of fecal results cannot be made at this point.

The information presented here suggests that low humidity, independent of diet or quantity of fecal material produced, results in the incorporation of significantly

TABLE 11 - Total radiolabelled fecal production in adult, male American cockroaches maintained on different 3 dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-glucose.

	LOW HUMIDITY[A] (25-35% RH)			MODERATE/HIGH HUMIDITY[B] (70-80% RH)		
	FW	OW	OO[1]	FW	OW	OO
FECES	(3) 26.0^{Aa} ± 9.6	(5) 5.2^{Ab} ± 1.2	--	(6) 95.3^{Bc} ± 18.4	(6) 4.0^{Bb} ± 0.7	(6) 6.2^{Bb} ± 1.2
DPM	(3) 19,889^{Aa} ± 2,449	(5) 12,824^{Aa} ± 1,406	--	(6) 8,791^{Bb} ± 785	(6) 7,461^{Bb} ± 2,066	(6) 17,136^{Aa} ± 1,935

FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
OO = no dog chow or water
FECES = average total fecal production in mg (15 d)
DPM = average DPM/average total fecal production (15d)
[1]No insects survived to 15 d
A,B = humidity levels produced significantly different values when tested using Duncans Multiple Range Test (alpha = 0.05)
Table values = MEAN ± SEM
(N) = number of insects
Means (in rows) with the same upper and lower case letters are not significantly different from each other (Standardized Students t-Test; alpha = 0,05)

more total radiolabel in the feces. A possible explanation may be that higher humidity would enhance the activity of intestinal bacteria that are eliminated with the feces resulting in more active degradation of radiolabelled fecal components than in the lower humidity. It should be mentioned, that tabulation of total radiolabel incorporated into feces reported here may not be complete for the dietary regimens. Possible residues in cages used in these experiments were not counted (only the fecal material which was collected) at the termination of the experiments, and may have contained a significant amount of radiolabel. Also, small quantities of fecal material were produced by both the OW and OO insects. Smaller fecal quantities would result in greater error if a portion of the sample were not collected and assayed. A combination of these factors could possibly affect the results in this section as well as those in Section 4.2.2 (Table 9). Future work should include methods which account for possible fecal/excretory residues, differences in total fecal production and effects attributable to humidity.

4.2.2.3 - $^{14}CO_2$ PRODUCTION

Information in Table 12 summarizes 15 d total $^{14}CO_2$ production in adult, male American cockroaches maintained

TABLE 12 - Total $^{14}CO_2$ production in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-glucose.

	LOW HUMIDITY [A] (25-35% RH)		
	FW	OW	OO
CO_2	(3) 1,648,936 ± 241,564A,a	(5) 2,042,230 ± 182,857A,a	(1) 849,175
	MODERATE/HIGH HUMIDITY [B] (70-80% RH)		
	FW	OW	OO
CO_2	(6) 2,003,426 ± 35,210B,a	(6) 2,030,482 ± 216,819B,a	(6) 593,923 ± 79,522B,b

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
CO_2 = average total $^{14}CO_2$ production (15 d)
Table values = MEAN ± SEM
(N) = number of insects
Means (within humidity levels) followed by the same upper and lower case letters are not significantly different
(Duncans Multiple Range Test, alpha = 0.05)

on different dietary regimens and held under 2 different humidity levels. Analysis indicated that $^{14}CO_2$ production over the 15 d experimental period was significantly different with regard to dietary regimens ($P < 0.0001$). The FW and OW insects produced similar quantities of $^{14}CO_2$ (FW low humidity = 1,648,936 DPM and moderate/high humidity = 2,003,426 DPM; OW low humidity = 2,042,230 DPM and moderate/high humidity = 2,030,482 DPM), and these insects in both humidity levels produced significantly more ($P < 0.05$) $^{14}CO_2$ than corresponding OO insects (OO low humidity = 849,175 DPM and moderate/high humidity = 593,923 DPM). Even though only 1 low humidity OO insect survived to 15 d, the value associated with this insect was comparable to that recorded for the 6 moderate/high humidity OO insects.

Information in Table 13 presents results on sequential $^{14}CO_2$ production by adult, male American cockroaches maintained on different dietary regimens and held under 2 different humidity levels after injection with radiolabelled glucose. Information in this table reflects average results discussed in Table 12 and clearly indicates that at 2 d, OO insects in both humidity levels produced significantly less ($P < 0.0001$) $^{14}CO_2$ (low = 78 DPM and moderate/high humidity = 97 DPM) than on either 1 d (low humidity = 313,720 DPM and moderate/high humidity = 32,343 DPM) or 4 d (low humidity = 193,356 DPM and moderate/high

TABLE 13 - Radiolabel as CO_2 released from adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-glucose.

LOW HUMIDITY (25-35% RH)

DAY	(N) FW	(N) OW	(N) OO
1	(6) 935,368	(6) 945,621	(6) 313,720
2	(6) 234,207	(6) 188,180	**(6) 78
4	(6) 272,325	(6) 207,767	(6) 193,356
6	(6) 138,896	(6) 136,335	(6) 151,837
8	(4) 95,781	(6) 417,394	(5) 101,346
11	(4) 78,809	(6) 99,426	(4) 57,651
13	(4) 43,978	(5) 72,214	*(1) 3,967
15	(3) 60,861	(5) 54,956	*(1) 12,496
MEAN	232,527 ± 104,513	265,237 ± 105,297	136,331 ± 45,070

MODERATE/HIGH HUMIDITY (70-80% RH)

DAY	(N) FW	(N) OW	(N) OO
1	(6) 756,268	(6) 713,225	(6) 32,343
2	(6) 289,704	(6) 271,655	**(6) 97
4	(6) 427,307	(6) 433,397	(6) 190,236
6	(6) 224,146	(6) 224,338	(6) 120,547
8	(6) 118,249	(6) 135,877	(6) 91,081
11	(6) 111,919	(6) 106,152	(6) 86,191
13	(6) 39,246	(6) 83,602	(6) 39,018
15	(6) 36,588	(6) 62,234	(6) 34,409
MEAN	250,428 ± 86,228	253,810 ± 78,591	74,240 ± 21,591

FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
OO = no dog chow or water
Table values = MEAN
(N) = number of insects
MEAN = MEAN ± SEM
* = value not used in AVG calculation due to low N
** value significantly different than other table values
(Duncans Multiple Range Test; alpha = 0.05)

sequestered radiolabelled materials and did not utilize them in catabolism resulting in little $^{14}CO_2$ release for the period of at least 1 d to 2d, and perhaps longer. The results strongly indicate that these cockroaches shifted their primary metabolic substrate from radiolabelled substrates to some other form. These data support the hypothesis that a change in substrate utilization may occur in cockroaches deprived of both food and water. Neither of the other 2 dietary regimens showed this same trend at 2 d (low humidity FW = 234,207 DPM and moderate/high humidity = 289,704 DPM; low humidity OW = 188,180 DPM and moderate/high humidity = 271,655 DPM).

Figures 7 and 8 show total $^{14}CO_2$ release over the 15 d experimental period. They indicate that terminal cumulative radiolabelled $^{14}CO_2$ for both humidities in OO insects (low humidity = 849,175 DPM and moderate/high humidity = 593,923 DPM) is significantly less ($P < 0.05$) than either FW or OW insects (FW low humidity = 1,648,936 DPM and moderate/high humidity = 2,003,426 DPM; OW low humidity = 2,042,230 DPM and moderate/high humidity = 2,030,482 DPM). In neither humidity level are the FW nor OW insects 15 d cumulative DPM as $^{14}CO_2$ different from each other. In addition, low humidity OW insects (Figure 7) show a sharp rise in $^{14}CO_2$ production from 6 d (1,477,904 DPM) to 8 d (1,895,298 DPM). This sharp rise in radiolabelled CO_2 is difficult to explain since the

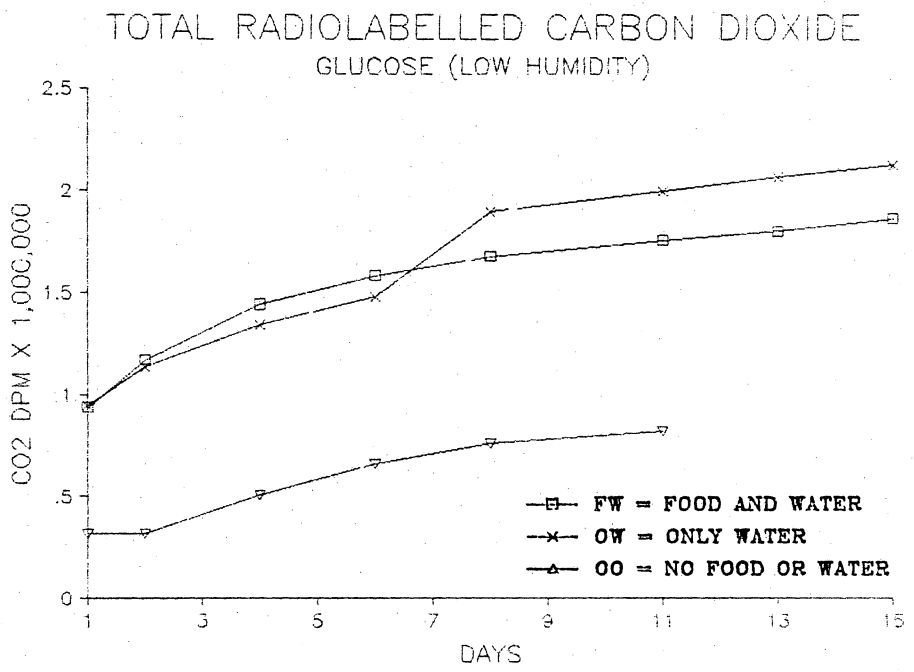


Figure 7. Cumulative $^{14}CO_2$ release from adult, male American cockroaches maintained on 3 different diets and low humidity (25-35% RH) after injection with ^{14}C-glucose.

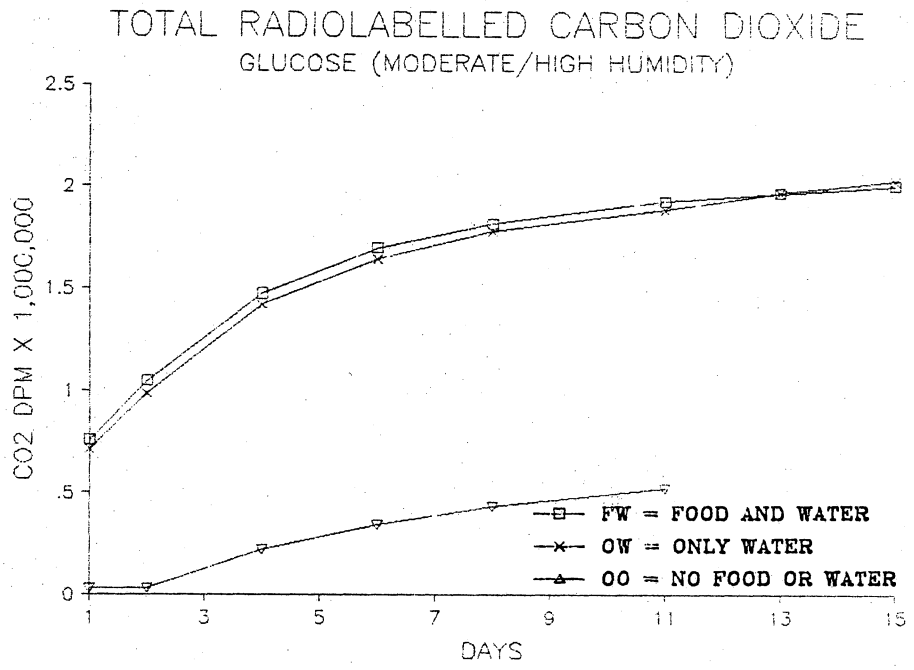


Figure 8. Cumulative $^{14}CO_2$ release from adult, male American cockroaches maintained on 3 different diets and moderate/high humidity (70-80% RH) after injection with ^{14}C-glucose.

radiolabelled CO_2 is difficult to explain since the corresponding RQ during this period was 0.75, indicative of lipid-based metabolism (refer to Section 4.1).

It is possible that the radiolabelled glucose was converted to lipid-based substrates prior to 6 d, and that the increase in radiolabel as $^{14}CO_2$ was the result of metabolism of these newly synthesized radiolabelled lipids. These results also support the hypothesis that cockroaches under dietary stress (food deprivation in this case) change their metabolic substrate utilization patterns.

4.2.3 - HEMOLYMPH

Information presented in Table 14 summarizes 15 d average radiolabel/ul of whole hemolymph resulting from ^{14}C-glucose injections in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels. Analysis of the whole hemolymph data indicated that there were no significant differences with respect to radiolabel/ul incorporation due to either dietary or humidity regimens. The information presented in this table suggests that slightly more radiolabel/ul was present in low humidity OW insects hemolymph (low humidity = 698 DPM and moderate/high humidity = 394 DPM) than any of the other dietary

TABLE 14 - Radiolabelled whole hemolymph in adult, male American cockroaches maintained on 3 different diets and held under 2 different humidity levels 15 d after injection with ^{14}C-glucose.[1]

	LOW HUMIDITY (25-35% RH)		
	FW	OW	OO
DPM/ul	(2) 288 ± 95	(5) 698 ± 230	--
	MODERATE/HIGH HUMIDITY (70-80% RH)		
	FW	OW	OO
DPM/ul	(6) 212 ± 21	(6) 394 ± 78	(6) 368 ± 78

[1]No significant differences were found when tested using Duncans Multiple Range Test (alpha = 0.05).
FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
DPM/ul = average DPM/ul whole hemolymph
Table values = MEAN
(N) = number of insects

regimens in either humidity level. In addition, more radiolabel/ul appeared in low humidity insect hemolymph than in moderate/high humidity insects. The latter 2 observations cannot be substantiated statistically due to overlap in range values. Even though the values presented in Table 14 are not significant, they suggest that insects deprived of food, but provided water, may be mobilizing radiolabelled fat body lipid reserves (refer to Section 4.2.2.3.), which could account for the increase in radiolabel seen in hemolymph samples. Also, it may be possible that low humidity OW insects, under somewhat greater stress to produce more metabolic water, incorporated slightly more radiolabel (possibly lipids) in the hemolymph than corresponding moderate/high humidity insects. In addition, wide range values for radiolabel observed in hemolymph samples in both humidity levels may be due, in part, to initial metabolic reserve differences in these insects at the initiation of the experiments. Downer (1985) suggested that cockroaches continually exhibit carbohydrate-lipid feedback cycles to regulate energy demands and that these cycles are responsive to dietary status. If this is true, then in these experiments the insects may not have been at the same point in their cycles at the initiation of these experiments, thus causing the observed differences.

4.2.4 - FAT BODY

Information presented in Table 15 summarizes 15 d radiolabel/mg of fat body tissue in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels after injection with ^{14}C-glucose. Information in this table indicates that low humidity insects had significantly higher ($P < 0.05$) radiolabel/mg fat body tissue (FW = 1,197 DPM/mg and OW = 1,745 DPM/mg) than corresponding moderate/high humidity insects (FW = 864 DPM/mg and OW = 966 DPM/mg). Although dietary regimens produced visually different quantities of fat body tissue, no significant differences in radiolabel /mg fat body tissue were observed at 15 d due to diet. Tucker (1977a) also noted variable fat body tissue in adult, male American cockroaches after 6 d of dehydration. In addition, low humidity OO insects, possibly under greater stress, may have utilized energy reserves sooner than corresponding moderate/high humidity insects, resulting in earlier mortality.

4.2.5 - CUTICLE AND ASSOCIATED MATERIALS

Information presented in Table 16 summarizes pronotal radiolabel and radiolabel contained in 1 ml volume of

TABLE 15 - Radiolabelled fat body tissue in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-glucose.

	LOW HUMIDITY[A] (25-35% RH)		
	FW	OW	OO
DPM/mg fat body	(3) 1,197 ± 303	(5) 1,745 ± 190	----

	MODERATE/HIGH HUMIDITY[B] (70-80% RH)		
	FW	OW	OO
DPM/mg fat body	(6) 864 ± 69	(6) 966 ± 105	(6) 1,349 ± 248

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
DPM/mg fat body = average DPM/mg fat body tissue (15 d)
Table values = MEAN ± SEM
(N) = number of insects
A,B = humidity levels produced significantly different values when tested using Duncans Multiple Range Test (alpha = 0.05)

TABLE 16 - Pronotal cuticle radiolabel and radiolabel released from pronotal cuticle tissue solublization obtained from adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-glucose.

	LOW HUMIDITY (25-35% RH)			MODERATE/HIGH HUMIDITY (70-80% RH)		
	FW	OW	OO	FW	OW	OO
PRO	(3) 28[a]	(4) 26[a]	--	(6) 93[a]	(6) 52[a]	(6) 23[a]
DPM/ml	(3) 1,169 ± 76[b]	(5) 1,580 ± 275[b]	(6) 1,463 ± 226[b]	(6) 1,169 ± 104[b]	(6) 1,564 ± 433[b]	(6) 1,462 ± 226[b]

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
PRO = average DPM/average pronotal weight (15 d)
DPM/ml = average DPM/ml tissue solublizer used to clear corresponding pronota
Table values = MEAN ± SEM
(N) = number of insects
Means followed by the same letter are not significantly different (Duncans Multiple Range Test, alpha = 0.05)

tissue solublizer (used to clear the pronota) obtained from adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels. Analysis showed no significant differences in radiolabel content in either pronotal samples or in respective 1 ml volumes of tissue solublizer used to clear the pronota due to dietary or humidity regimens. Information in this table suggests that tissue solublizing media contained more radiolabel than corresponding pronotal samples (FW - low humidity = 41.8 X and moderate/high humidity = 12.6 X; OW - low humidity = 60.8 X and moderate/high humidity = 30.1 X; OO - low humidity = pronotal samples below background and moderate/high humidity = 63.6 X). Although there was a significant difference ($P < 0.05$) in radiolabel content in the tissue solublizing media (used to clear the pronota) and the cleared pronota, no conclusions can be obtained from these results. This is because information obtained may reflect variability in the pronotal rinsing technique itself or represent cuticular contamination due to unidentifiable components. Also, the low pronotal radiolabel content (near background) was not large enough to consider using the tissue solublizer radiolabel content to determine cuticular component incorporation.

4.3 - ^{14}C-PALMITIC ACID EXPERIMENTS

Preliminary analysis of the data from 3 radiolabel palmitic acid experiments indicated that, as in the Respirometric Study (Section 4.1) and the radiolabel glucose experiments (Section 4.2.1), humidity did not significantly influence radiolabel incorporation into whole body, hemolymph, fat body tissue and cuticle and associated cuticular materials. However, feces and $^{14}CO_2$ production did show significantly different results ($P < 0.05$) in insects held at the 2 different humidity levels (low= 25-35% and moderate/high=70-80% RH). Because of this, most tables will include information obtained from both humidity levels for comparative purposes. In the radiolabel palmitic acid experiments, more low humidity cockroaches survived to 15 d than those in the radiolabel glucose experiments.

4.3.1 - OVERVIEW

Information presented in Table 17 summarizes results of 15 d total radiolabel incorporation into whole body, feces and CO_2 compared to both the average zero time whole body (control insects) radiolabel recorded for each dietary

TABLE 17 - Radiolabel incorporation in whole body, feces and CO_2 as compared to average zero time insects in adult, male American cockroaches maintained on 3 different dietary regimens 15 d after injection with ^{14}C-palmitic acid.

	DPM		
	FW	OW	OO
T(0)	(4) 341,554 ± 26,934	(4) 407,637 ± 6,141	(4) 418,739 ± 21,448
T(IC)	(6) 590,489 ± 21,593	(8) 587,611 ± 17,772	(8) 641,521 ± 3,761
% REC	57.8	69.4	65.3
WB	(12) 202,783 ± 16,464[a]	(12) 260,382 ± 22,562[b]	(10) 266,592 ± 21,929[b]
FECES	(12) 4,375 ± 480	(12) 4,630 ± 1,659	(10) 6,194 ± 79
CO_2	(12) 252,435 ± 23,177a	(12) 290,081 ± 5,858b	(10) 260,301 ± 34,740a
TOTAL	459,593	555,093	533,087
% T(0)	135	136	127
% T(IC)	78	95	83

FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
OO = no dog chow or water
T(0) = average DPM/average body weight recovered from zero time insects
T(IC) = average DPM/(N) applications to filter paper strips before, during and after insect injections of ^{14}C-palmitic acid
% REC = T(0)/T(IC) X 100
WB = average DPM/average body weight (15 d)
FECES = average DPM/average total fecal production
CO_2 = average total $^{12}CO_2$ produced (15 d)
TOTAL = WB + FECES + CO_2
% T(0) = TOTAL/T(0)
% T(IC) = TOTAL/T(IC)
Table values = MEAN ± SEM
(N) = number of insects
Means (within rows) followed by the same letter are not significantly different (Duncans Multiple Range Test, alpha = 0.05) fecal

regimen after injection with ^{14}C- palmitic acid and theoretical injected amounts. Information presented in Table 17 indicates that 15 d insect whole bodies contained significantly different ($P < 0.05$) levels of total radiolabel among dietary regimens. The OO insects retained significantly more ($P < 0.05$) radiolabel/insect (266,592 DPM) than FW insects (202,783 DPM), but similar amounts to those recorded for OW insects (260,382 DPM). The OO insects appeared to have a higher fecal radiolabel incorporation (6,194 DPM), but this was not significantly different from either FW (4,375 DPM) or OW (4,630 DPM) insects. As reported in Section 4.2.2, a major effort was not made to assure that radiolabel did not remain with fecal residues in individual cages after feces were collected at 15 d. This could represent a source of some unaccountable radiolabel. The OW insects released significantly more ($P < 0.05$) radiolabelled $^{14}CO_2$ (290,081 DPM) than either FW (252,435 DPM) or OO (260,301 DPM) insects. If OW insects were metabolizing lipids heavily, as suggested by RQ data (Section 4.1), one would expect to observe significantly more radiolabel released as CO_2 as well as a low WB radiolabel in OW insects than in either FW or OO insects (RQ data for the FW and OO insects agree with this assumption - see Section 4.1).

Comparisons of zero time whole body (injected controls) radiolabel recovery with theoretical injected

amounts (IC) indicated that recovery of radiolabel was highest in OW insects (69.4%), followed by OO (65.3%) and FW (57.8%) insects. The relationship of radiolabel recovery percentages was the same in both the glucose and palmitic acid studies with greatest recovery in OW insects, followed by OO and FW insects. In addition, these low recoveries from zero time (injected controls) insects when compared to total recovery in 15 d whole body, feces and CO_2 may explain the high percentages recorded for % T(0) in dietary regimens (FW = 135%, OW = 136% and OO = 127%). Comparisons of total radiolabel in 15 d whole body, feces and CO_2 to theoretical injection values resulted in lower percentages (FW = 78%, OW = 95% and OO = 83%). These results indicate that recovery in zero time insects (injected controls) may be significantly lower than actual injected quantities. Specific information on whole body, feces and CO_2 are reported below. These results indicate that insects deprived of food and/or water (OW and OO insects) appear to retain greater quantities of radiolabelled material for extended periods. As previously mentioned, higher radiolabel content occurred in feces when cockroaches were deprived of food and/or water (OW = 4,630 DPM and OO = 6,194 DPM) compared to content in controls (FW = 4,375 DPM), but interpretation of the fecal information remains incomplete.

4.3.2 - WHOLE BODY

Information presented in Table 18 summarizes 15 d average whole body weights, radiolabel/insect, and radiolabel/zero time whole body (control insects) and estimated theoretical injections in adult, male American cockroaches maintained on 3 different dietary regimens after injection with radiolabelled palmitic acid. Preliminary statistical analysis indicated that dietary regimens produced significant differences in respect to incorporation of radiolabel in 15 d insect whole bodies ($P < 0.0409$). Further analysis indicated that FW insects (202,783 DPM), at 15 d, retained significantly less ($P < 0.05$) radiolabel than either OW or OO insects (260,382 DPM and 266,592 DPM, respectively). Values obtained from OW and OO insects were not significantly different from each other.

The fact that OW insects exhibited an RQ indicative of lipid metabolism at 15 d (refer to Section 4.1) suggests these insects should have lower 15 d whole body radiolabel content than either FW or OO insects. Considering that the QO_2 of OW insects (refer to Section 4.1.2) toward the end of the 15 d experimental period was the lowest recorded for the dietary regimens suggests that even though lipids may have been the primary metabolic substrate, their

TABLE 18 - Whole body weights, total radiolabel/insect and radiolabel/zero time insect in adult, male American cockroaches maintained on 3 different dietary regimens 15 d after injection with ^{14}C-palmitic acid.

	FW	OW	OO
BW	(12) 723 ± 34	(12) 726 ± 25	(10) 603 ± 21
TOTAL DPM/INSECT	(12) 202,783 ± 16,464[a]	(12) 260,382 ± 22,562[b]	(10) 266,592 ± 21,929[b]
DPM INJ (CONTROL)	(4) 341,554 ± 26,934	(4) 407,637 ± 6,141	(4) 418,739 ± 21,448
INSECT %	59.4%	63.9%	63.7%
(IC)	(6) 590,489 ± 21,593	(8) 587,611 ± 17,772	(8) 641,521 ± 3,761

FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
OO = no dog chow or water
BW = average wet body weight (15 d)
TOTAL DPM/INSECT = average DPM/average wet body weight (15 d)
DPM INJ (CONTROL) = average DPM recovered from zero time insects/average body weight
INSECT % = (TOTAL DPM/INSECT)/DPM INJ (CONTROL)
(IC) = average DPM/(N) applications to filter paper strips before, during and after insect injections of radiolabelled palmitic acid
Table values = MEAN ± SEM
(N) = number of insects
Means followed by the same letter are not significantly different (Duncans Multiple Range Test; alpha = 0.05)

utilization rate was much slower. It should be noted that RQ values for OO insects also did not reflect lipid substrate utilization, and in fact these insects did have a higher 15 d whole body radiolabel content than either the FW or OW insects even though they exhibited the highest QO_2 of the dietary regimens.

4.3.3 - FECES

Information presented in Table 19 summarizes 15 d total feces (mg) and radiolabel incorporation into feces in adult, male American cockroaches maintained on 3 different diets and under 2 different humidity levels. As reported in the radiolabel glucose experiments (Section 4.2.1), incorporation of radiolabel into feces was significantly affected by humidity levels ($P < 0.025$) in these experiments. Dietary regimens were not significant in producing differences in radiolabel incorporation into feces (FW - low humidity = 4,855 DPM and moderate/high humidity = 3,895 DPM; OW - low humidity = 6,289 DPM and moderate/high humidity = 2,971 DPM; OO - low humidity = 6,273 DPM and moderate/high humidity = 6,115 DPM). As mentioned in Section 4.3.1, the higher humidity may have enhanced bacterial degradation of the ^{14}C- products in the feces more than in the lower humidity. Also,

TABLE 19 - Total radiolabelled fecal production (mg) in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-palmitic acid.

	LOW HUMIDITY[A] (25-35% RH)		
	FW	OW	OO
FECES	(6) 29.0 ± 9.3^{Aa}	(6) 4.8 ± 0.6^{Ab}	(4) 4.5 ± 0.3^{Ab}
DPM	(6) 4,855 ± 578	(6) 6,289 ± 417	(4) 6,273 ± 428
	MODERATE/HIGH HUMIDITY[B] (25-35% RH)		
	FW	OW	OO
FECES	(6) 35.7 ± 7.4^{Ba}	(6) 3.8 ± 1.1^{Bb}	(6) 7.2 ± 0.9^{Bb}
DPM	(6) 3,895 ± 4,473	(6) 2,971 ± 803	(6) 6,115 ± 618

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
FECES = average total fecal production (15 d)
DPM = average DPM/average total fecal production (15 d)
Table values = MEAN ± SEM
(N) = number of insects
A,B = differences in humidity produced significantly different values (Duncans Multiple Range Test; alpha = 0.05)
Means followed by the same upper and lower case letters are not significantly different (Duncans Multiple Range Test; alpha = 0.05)

fecal/excretory residues of insect cages at 15 d were not assayed, therefore significantly more radiolabel may have been associated with fecal samples. In addition, significantly more ($P < 0.05$) fecal material was produced by FW insects in both low and moderate/high humidity levels (low humidity = 29.0 mg and moderate/high humidity = 35.7 mg) than was produced in both humidities by either OW or OO insects (OW low humidity = 4.8 mg and moderate/high humidity = 3.8 mg; OO low humidity = 4.5 mg and moderate/high humidity = 7.2 mg).

4.3.4 - $^{14}CO_2$ PRODUCTION

Information presented in Table 20 summarizes 15 d total $^{14}CO_2$ in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels. The data suggest that moderate/high humidity 15 d total $^{14}CO_2$ production is similar among diets, but that total radiolabelled $^{14}CO_2$ was significantly higher ($P < 0.05$) in moderate/high humidity insects (FW = 275,258 DPM, OW = 295,939 DPM and OO = 295,041 DPM) than in low humidity insects (FW = 229,258 DPM, OW = 284,222 DPM and OO = 225,561 DPM). The low humidity FW and OO insects did not indicate any significant difference in total $^{14}CO_2$ due to dietary regimens, but were significantly lower than

TABLE 20 - Total $^{14}CO_2$ production in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-palmitic acid.

	LOW HUMIDITY[A] (25-35% RH)		
	FW	OW	OO
CO_2	(6) 229,258 ± 3,670A,a	(6) 284,222 ± 23,409A,b	(4) 225,561 ± 24,296A,a
	MODERATE/HIGH HUMIDITY[B] 70-80% RH)		
	FW	OW	OO
CO_2	(6) 275,612 ± 20,677B,a	(6) 295,939 ± 30,077B,a	(6) 295,041 ± 18,408B,a

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
CO_2 = average total $^{14}CO_2$ produced (15 d)
Table values = MEAN ± SEM
(N) = number of insects
A,B = humidity produced significantly different results using Duncans Multiple Range Test (alpha = 0.05)
Means (within rows) followed by the same upper and lower case letters are not significantly different (Duncans Multiple Range Test; alpha = 0.05)

low humidity OW insects ($P < 0.05$). This suggests that low humidity OW insects possibly were utilizing more lipids as metabolic substrates, and agrees with RQ data (Section 4.1.1). The 15 d whole body radiolabel/insect data (Section 4.3.2) suggest that the OW insects retained more radiolabel than FW insects and less than OO insects. Since QO_2 data (Section 4.1.2) suggest that metabolism in the OW insects has been slowed, these insects may be utilizing primarily lipid substrates at slower rates.

Information presented in Table 21 summarizes 15 d cumulative $^{14}CO_2$ in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels after injections of radiolabelled palmitic acid. Information from this table reflects similar relationships found in 15 d total $^{14}CO_2$ data (refer to Table 20) and suggests that low humidity insects appeared to produce slightly less average radiolabelled CO_2 (FW = 28,657 DPM, OW = 35,535 DPM and OO = 27,228 DPM) than insects in the corresponding dietary regimens in moderate/high humidity (FW = 34,452 DPM, OW = 36,993 DPM and OO = 36,880 DPM), even though these values were not significantly different from each other. This decreased $^{14}CO_2$ production may be in response to decreased low humidity insect activity (Tucker, 1977a).

Average cumulative $^{14}CO_2$ release over 15 d experimental periods are presented in Figures 9 and 10 for

TABLE 21 - Radiolabel as $^{14}CO_2$ released form adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-palmitic acid. [1]

LOW HUMIDITY (25-35% RH)

DAY	FW	OW	OO
1	(6) 53,555	(6) 58,914	(6) 67,061
2	(6) 42,230	(6) 32,198	(6) 34,410
4	(6) 47,479	(6) 58,216	(6) 36,329
6	(6) 26,829	(6) 36,339	(6) 20,054
8	(6) 18,306	(6) 22,177	(6) 16,618
11	(6) 21,164	(6) 30,126	(5) 22,503
13	(6) 9,516	(6) 20,421	(5) 9,341
15	(6) 10,180	(6) 25,890	(4) 11,505
OVERALL MEAN	28,657 ± 6,025	35,535 ± 5,348	27,228 ± 6,648

MODERATE/HIGH HUMIDITY (70-80% RH)

DAY	FW	OW	OO
1	(6) 65,832	(6) 73,420	(6) 78,643
2	(6) 43,073	(6) 27,498	(6) 46,368
4	(6) 58,529	(6) 61,235	(6) 59,950
6	(6) 33,373	(6) 43,043	(6) 30,464
8	(6) 24,260	(6) 24,648	(6) 27,066
11	(6) 26,197	(6) 30,360	(6) 29,633
13	(6) 12,726	(6) 17,271	(6) 12,010
15	(6) 11,622	(6) 18,466	(6) 10,907
OVERALL MEAN	34,452 ± 7,078	36,993 ± 7,283	36,880 ± 8,291

[1]No significant differences found when tested using Duncans Multiple Range Test (alpha = 0.05)
FW = dog chow and distilled water ad libitum
OW = distilled water ad libitum
Table values = MEAN
(N) = number of insects
OVERALL MEAN = MEAN ± SEM

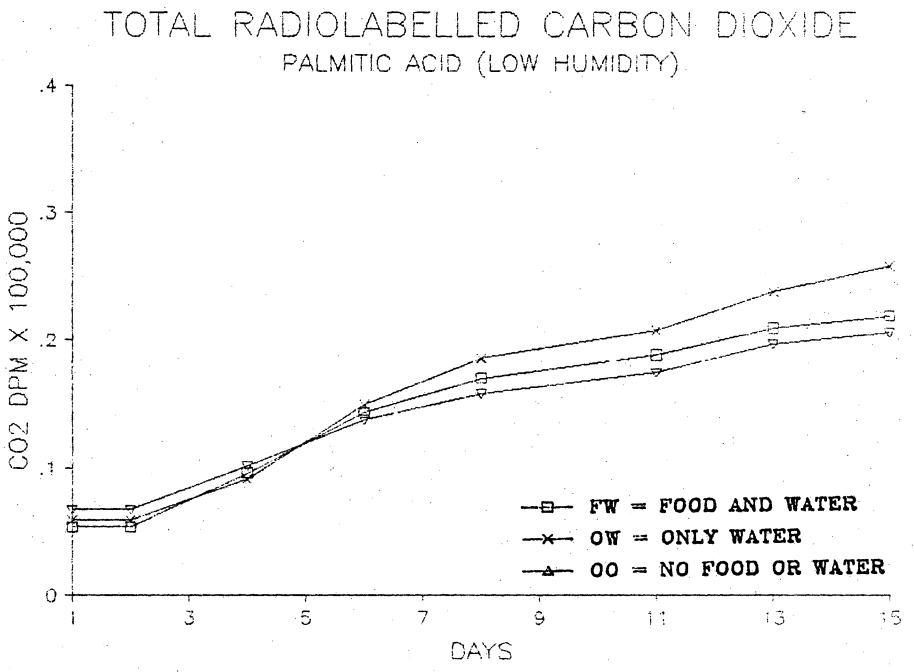


Figure 9. Cumulative $^{14}CO_2$ release from adult, male American cockroaches maintained on 3 different diets and low humidity (25-35% RH) after injection with ^{14}C-palmitic acid.

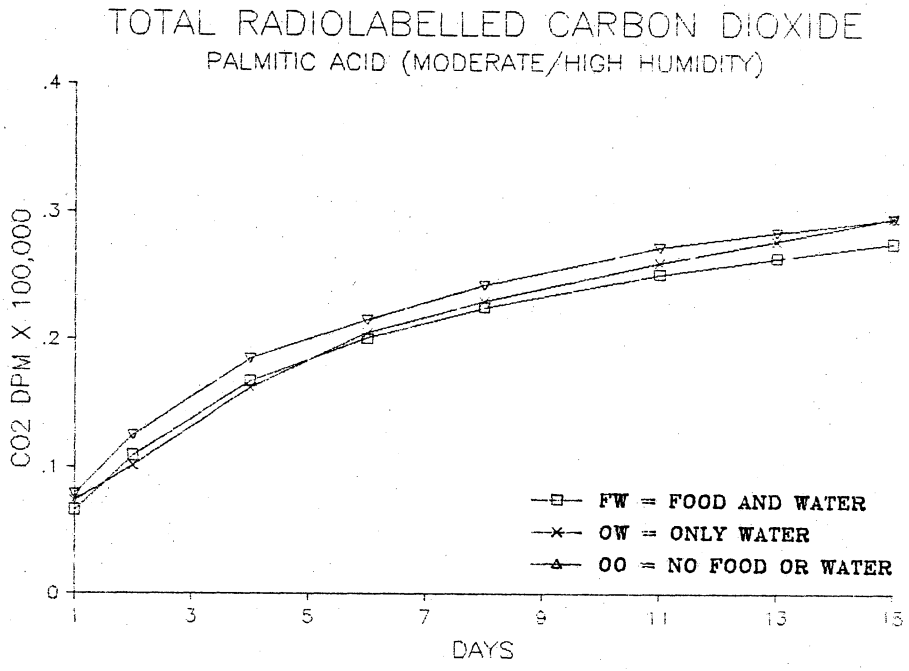


Figure 10. Cumulative $^{14}CO_2$ release from adult, male American cockroaches maintained on 3 different diets and moderate/high (70-80% RH) after injection with ^{14}C-palmitic acid.

low and moderate/high humidities, respectively. These figures depict information discussed previously in Tables 20 and 21 and indicate that average cumulative $^{14}CO_2$ rose steadily over the experimental period within dietary regimens in both humidity levels. The moderate/high humidity insects (Figure 10) produced significantly more ($P < 0.05$) radiolabelled CO_2 than did low humidity insects (Figure 9), possibly the result of consistently higher $^{14}CO_2$ production in all 3 dietary regimens within moderate/high humidity regimen.

4.3.5 - HEMOLYMPH

Information presented in Table 22 summarizes 15 d radiolable/ul whole hemolymph data resulting from radiolabelled palmitic acid injections in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels. Information in this table indicates that humidity levels were not significant factors in producing differences in radiolabel/ul hemolymph, but that dietary regimens (within humidity levels) were significant ($P < 0.05$) in producing differences in radiolabel/ul hemolymph. Low humidity insects (on average) contained higher radiolabel/ul of hemolymph (FW = 73 DPM, OW = 90 DPM and OO = 115 DPM) compared to moderate/high humidity insects (FW = 69 DPM, OW

TABLE 22 - Radiolabelled whole hemolymph in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-palmitic acid.

	LOW HUMIDITY (25-35% RH)		
	FW	OW	OO
DPM/ul	(6) 73 ± 8[a]	(6) 90 ± 7[a]	(3) 115 ± 25[b]
	MODERATE/HIGH HUMIDITY (70-80% RH)		
	FW	OW	OO
DPM/ul	(6) 69 ± 9[a]	(6) 64 ± 8[a]	(6) 95 ± 12[b]

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
DPM/ul = average DPM/ul whole hemolymph (15 d)
Table values = MEAN ± SEM
(N) = number of insects
Means (within rows) followed by the same letter are not significantly different (Duncans Multiple Range Test; alpha = 0.05)

= 64 DPM and OO = 95 DPM). Since low humidity insects and moderate/high OO insects have greater water requirements than insects maintained under other experimental conditions, these insects may be mobilizing lipids in an attempt to gain more needed metabolic water.

Unlike results in the glucose experiments (Section 4.2.3), diet significantly affected radiolabel/ul whole hemolymph at 15 d ($P < 0.0132$) in the radiolabel palmitic acid experiments (compare Tables 14 and 22). The OO insects held under both humidities contained significantly more ($P < 0.05$) radiolabel/ul whole hemolymph at 15 d than did either FW or OW insects. Currently, the biochemical sequence of events leading to the increase in radiolabel in OO insect hemolymph is unknown, but it may include the mobilization of fat body tissue in response to antidiuretic factors released (Hyatt and Marshall, 1985). There were no significant differences between hemolymph radiolabel/ul in FW and OW insects (within each humidity level), but both were significantly less ($P < 0.05$) than corresponding OO insects in both humidity levels at 15 d.

4.3.6 - FAT BODY

Information presented in Table 23 summarizes 15 d radiolabel/mg fat body tissue in adult, male American cockroaches maintained on 3 different dietary regimens

TABLE 23 - Radiolabelled fat body tissue in adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-palmitic acid.[1]

	LOW HUMIDITY (25-35% RH)		
	FW	OW	OO
DPM	(6) 272 ± 36	(6) 456 ± 137	(6) 999 ± 283
	MODERATE/HIGH HUMIDITY (70-80% RH)		
	FW	OW	OO
DPM	(6) 218 ± 27	(6) 1305 ± 847	(6) 897 ± 179

[1]No significant differences were found when tested using Duncans Multiple Range Test (alpha = 0.05)
FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or distilled (15 d)
DPM = average DPM/mg fat body
Table values = MEAN ± SEM
(N) = number of insects

and held under 2 different humidity levels after injection with ^{14}C-palmitic acid. Information presented in Table 23 suggests that in both humidity levels FW insect fat body tissue appeared to contain less radiolabel (low humidity = 272 DPM and moderate/high humidity = 218 DPM) than either OW (low humidity = 456 DPM and moderate/high humidity = 1305 DPM) or OO (low humidity = 999 DPM and moderate/high humidity = 897 DPM) insects, but no significant differences were found in radiolabel/mg fat body due to either humidity or dietary regimens. This lack of significance can be most likely attributed to the overlap within dietary regimen ranges in respect to radiolabel/mg fat body which was so large that it diminished the ability of the statistical test to establish significance. However, these results support findings reported in Section 4.2.4 (Table 15) in that FW insects in both humidity levels in the glucose experiment had lower 15 d radiolabel/mg fat body than either OW or OO insects. In both the glucose and palmitic acid experiments fat body quantity varied among experimental groups, and analysis indicated no significant differences in radiolabel/mg of tissue due to dietary regimens. Although in the glucose experiments, humidity significantly affected radiolabel incorporation into fat body tissue.

4.3.7 - CUTICLE AND ASSOCIATED MATERIALS

Information contained in Table 24 summarizes pronotal and radiolabel contained in 1 ml volume of tissue solubilizer obtained from adult, male American cockroaches maintained on 3 dietary regimens and held under 2 different humidity levels. Information in this table suggests that there were no significant differences in radiolabel content in either pronotal or tissue solublizer samples due to humidity. Also, no significant differences occurred in pronotal samples due to dietary regimens, but dietary regimens appeared to significantly affect ($P < 0.05$) radiolabel content in tissue solublizer samples. In both humidity levels, OO insects (low humidity = 517 DPM and moderate/high humidity = 472 DPM) appeared to incorporate significantly more ($P < 0.05$) radiolabel into tissue solublizing media than FW (low humidity = 373 DPM and moderate/high humidity = 363 DPM) insects. Low and moderate/high humidity OW insects showed no significant differences in respect to radiolabel content in tissue solublizing media to quantities recorded in either FW or OO insects.

These results suggest that the tissue solubilizing media had more radiolabel than corresponding pronotal samples (FW low humidity = 53.2 X and moderate/high

TABLE 24 - Pronotal cuticle radiolabel and radiolabel released from pronotal cuticle tissue solublization obtained from adult, male American cockroaches maintained on 3 different dietary regimens and held under 2 different humidity levels 15 d after injection with ^{14}C-palmitic acid.

	LOW HUMIDITY (25-35% RH)		
	FW	OW	OO
DPM/PRO	(6) 7 ± 2	(6) 18 ± 11	(3) 15 ± 10
DPM/ml	(6) 373 ± 10[a]	(6) 437 ± 61[a,b]	(4) 517 ± 99[b]
	MODERATE/HIGH HUMIDITY 70-80% RH)		
	FW	OW	OO
DPM/PRO	(6) 9 ± 2	(6) 10 ± 2	(3) 10 ± 3
DPM/ml	(6) 363 ± 19[a]	(6) 453 ± 53[a,b]	(6) 472 ± 23[b]

FW = dog chow and distilled water ad libitum (15 d)
OW = distilled water ad libitum (15 d)
OO = no dog chow or water (15 d)
DPM/PRO = average DPM/average pronotal weight (15 d)
DPM/ml = average DPM/ml corresponding tissue solubelizing media
Table values = MEAN ± SEM
(N) = number of insects
Means (within rows) followed by the same letter are not significantly different (Duncans Multiple Range Test, alpha = 0.05)

humidity = 40.3 X; OW low humidity = 24.3 X and moderate/high humidity = 45.3 X; OO low humidity = 34.5 X and moderate/high humidity = 47.2 X). These values may reflect variability in pronotal rinsing technique and/or possible contamination of cuticular samples. Again, as stated in Section 4.2.5, cuticular radiolabel content is so near background that no conclusions can be made concerning these data without further study.

5.0 - GENERAL DISCUSSION/CONCLUSIONS

This work was designed to test the hypothesis that American cockroaches, Periplaneta americana, when held under varying relative humidities and deprived of food and/or water, for a period of time, would change their initial metabolic utilization pattern from typical carbohydrate-based substrates to lipid-based substrates to supplement their energy and water requirements. It is not clear from the literature whether these changes do indeed occur, or if they do, what circumstances surround the requirements for these possible changes in metabolic substrate utilization patterns. Some workers (Tucker, 1977a and Wall, 1970) have suggested that changes in metabolic substrate utilization patterns are possible in Periplaneta americana and may occur when they are deprived of food and/or water. Machin (1981) has suggested that if these changes occur they would be counterproductive in terms of the amount of metabolic water produced compared to the energy expended by the insect to produce this additional metabolic water. This study was conducted to determine if changes in metabolic utilization patterns in Periplaneta americana do occur, and to what extent specific factors were responsible for these changes.

Three studies (respirometric, ^{14}C-glucose and ^{14}C-palmitic acid metabolism) were designed to determine the effects of diet and humidity on specific physiological processes, tissues, excreta and the insect as a whole. Information presented in Table 25 provides a comparison of insect responses, experimental vs control, in adult, male American cockroaches under varying conditions of diet and humidity in the respirometric, ^{14}C-glucose and ^{14}C-palmitic acid studies. Information in Table 25 relates the physiological and metabolic responses of experimental dietary regimens under varying conditions of relative humidity to control insect responses. This table is meant to provide an overall summary of physiological and metabolic responses. A detailed discussion involving results in each of the dietary regimens follows and focuses on the specific characteristics of each and the apparent cause.

CONTROL INSECTS (FW)

Control insects (FW), after 15 d of confinement, showed little overall change in QO_2, food consumption and terminal body weights. Except for the low initial RQ (0.74) recorded in the medium humidity controls and its subsequent rise (to 0.87), RQ values (mean = 0.83 $\pm$ 0.01)

TABLE 25 - Overall comparison of experimental adult, male American cockroach responses to control insect responses[1] under experimental conditions in the respirometric, ^{14}C-glucose and ^{14}C-palmitic acid studies.

RESPIROMETRIC STUDY								
	LH				MH			
	FW	OW	FO	OO	FW	OW	FO	OO
RQ	O	D*	I	I	O	D*	D	--
QO_2	O	D*	I	I*	O	D	I*	I*
FOOD	O	NA	D*	NA	O	NA	D*	NA
WATER	O	I	NA	NA	O	D	NA	NA
BW	O	I	D*	D*	O	D	D*	D*
BWC	O	D	D*	D*	O	D	D*	D*

	^{14}C-GLUCOSE STUDY						^{14}C-PALMITIC ACID STUDY					
	LH			M/H			LH			M/H		
	FW	OW	OO	FW	OW	OO	FW	OW	OO	FW	OW	OO
$^{14}CO_2$	O	I	NA	O	I	D*	O	I*	D	O	I	I
FECES	O	D*	NA	O	D*	D*	O	D*	D*	O	D*	D*
FECES/DPM	O	D	NA	O	D	I*	O	I	I	O	D	I
WB	O	I	NA	O	I	I	O	I*	I*	O	I*	I*
HEMOLYMPH	O	I	NA	O	I	I	O	I	I*	O	D	I*
FAT BODY	O	I	NA	O	I	I	O	I	I	O	I	I
CUTICLE	O	D	NA	O	D	D	O	I	I	O	I	I*

[1] control insects = O; I = increase compared to control; D = decrease compared to control; -- = no different from control (all table entries represent final experimental determinations; * = statistically significant; NA = not applicable)

LH = (25-35% RH); MH = (55-65% RH); M/H = (70-80% RH)

FW = food and water ad libitum; OW = distilled water ad libitum; FO = food only ad libitum; OO = no food or water

RQ = respiratory quotient

QO_2 = oxygen quotient

FOOD = average daily food consumption (mg)

WATER = average daily water consumption (ul)

BW = body weight (mg)

BWC = body weight change from original weight

$^{14}CO_2$ = total radiolabelled CO_2

FECES = total fecal material (mg)

FECES/DPM = radiolabel/total fecal production

WB = radiolabel/whole body

HEMOLYMPH = radiolabel/ul whole hemolymph

FB = radiolabel/mg fat body tissue

CUTICLE = radiolabel/pronotum

were equivalent to those recorded for insects under similar conditions (i.e. Tucker, 1977a). QO_2 values (mean = 0.38 $\pm$ 0.01 were higher than some reported for *P. americana* studied for shorter periods (Banks *et al.*, 1975); QO_2 values in the present study were recorded over a 15 d period. These extended observational periods may provide more representative QO_2 values than those previously reported.

Food consumption in the control insects at medium humidity (29.4 $\pm$ 3.5 mg/d) was similar to values reported by Hyatt and Marshall (1985) of 44.6 $\pm$ 11.5 mg/d. The insects at low humidity appeared to eat less, but the difference was not found to be significant.

From the original hypothesis, it was anticipated that water consumption rates in the low humidity control insects would be higher than those of the medium humidity control insects because of a greater expected water requirement, likely due to increased transpiration rates. The opposite was found to be the case. These results are difficult to explain and similar results have not appeared in the literature to date. Confinement under low humidity may reduce physical activity, thus feeding and drinking, as suggested by Tucker (1977a). It is also possible that activity may have been reduced by desiccation (Reynierse *et al.*, 1972). In addition, exposure of these insects to a continuous air stream may have increased transpiration

rates, thus affecting these results. Regardless of the specific factor(s) involved, the low humidity control insects did imbibe less water (on average) than the medium humidity control insects.

In the ^{14}C-glucose study, control insects showed little overall significant difference in the parameters examined with respect to the humidity levels imposed. Significant differences in radiolabel content, attributable to humidity, were observed in the feces and fat body tissue in this study, but $^{14}CO_2$ release and radiolabel content of whole body, hemolymph and cuticle samples did not show any significant differences. The significant differences found in fecal material radiolabel may not have been related to physiological processes, but may have been attributable to the effect of environmental factors after the fecal material was eliminated.

Control insects in the ^{14}C-glucose experiments in the moderate/high humidity produced significantly more fecal material which contained significantly less radiolabel than that of the low humidity insects. As mentioned previously in the results section, the higher humidity may have enhanced bacterial degradation of the eliminated radiolabelled fecal products. Definitive interpretation of fecal data is difficult under the present experimental conditions.

Fat body tissue of low humidity control insects in the ^{14}C- glucose study contained significantly more radiolabel than that of the moderate/high humidity insects. This may be due, in part, to mobilization of fat body water reserves in response to greater water requirements, created by the lower humidity environment.

The ^{14}C-palmitic acid study produced results similar to those of the ^{14}C-glucose study in that humidity levels were not significant factors in causing major differences in radiolabel content in most parameters examined. Humidity affected total fecal production and $^{14}CO_2$ release, but not radiolabel content in whole body, hemolymph, fat body tissue or cuticular samples.

In the ^{14}C-palmitic acid study radiolabel content of the feces from insects in both humidity levels were not significantly different, but moderate/high humidity insects produced more feces than the low humidity insects. This may suggest that the eliminated ^{14}C-materials may be affected less by humidity than those eliminated in the glucose study, or that bacteria eliminated with the moderate/high humidity insect feces more actively metabolize radiolabelled materials produced from carbohydrate precursors. Future studies need to include methods which will identify differences in radiolabelled fecal materials and studies of the associated bacteria.

METABOLIC AND PHYSIOLOGICAL RESPONSES OF STARVED COCKROACHES

The starved (OW) insects (provided only distilled water ad libitum) showed significantly lower terminal RQ and QO_2 values, after 15 d of confinement. The RQ data are consistent with the literature as both Tucker (1977a) and Wall (1970) observed a similar change in substrate metabolism (due to starvation) from carbohydrate-based to lipid-based substrates. QO_2 data recorded under these conditions are difficult to interpret. It was expected that these insects, apparently metabolizing lipids, would have had higher oxygen consumption rates than control insects.

Body weight of OW insects did not change significantly over the experimental period, but did show a slight reduction possibly associated with utilization of body reserves when results of both the humidities were combined. Also, an increase in total body water (compared to the controls) was noted. These results are consistent with those of Wharton et al. (1965), Wall (1970) and Tucker (1977a) in that starved insects do not show significant decreases in total body weight, and that starvation increases the percent water content of the cockroach in relation to its weight.

Water consumption in the low humidity starved (OW) insects fluctuated somewhat, but showed a continuous rise throughout the experimental period, while the medium humidity starved insects tended to have a more cyclic consumption. This cyclic consumption was responsible for the significant difference associated with daily water consumption among the medium humidity insects.

Whole body radiolabel was slightly higher at 15 d in starved (OW) insects than in control (FW) insects in the ^{14}C-glucose study and significantly higher in the ^{14}C-palmitic acid study. This suggests that an overall, slower rate of radiolabel metabolism (supported by QO_2 data) may have occurred, while an increase in lipid utilization may occur (supported by RQ data).

In both the ^{14}C-glucose and ^{14}C-palmitic acid studies total fecal production in starved (OW) insects was significantly less than that of control (FW) insects. These results agree with those of Wall (1970) in that fecal production is reduced 3 - 4 d after food deprivation, and during this period gut contents are eliminated. Also, in both radiolabel studies fecal radiolabel content in low humidity starved (OW) insects was significantly higher than in the moderate/high humidity starved insects, again suggesting the bacteria associated with eliminated feces may have been more active in the higher humidity environment. Although, no fecal comparisons of dehydrated

and starved low humidity insects and moderate/high humidity dehydrated and starved insects could be made (due to death of all low humidity OO insects) the moderate/high humidity dehydrated and starved insects had fecal radiolabel content similar to that found in both moderate/high humidity control and starved insects.

In the ^{14}C-glucose study, total $^{14}CO_2$ released by starved (OW) insects was not significantly different between the 2 humidity levels. In the ^{14}C-palmitic acid study, moderate/high humidity insects (in general) released significantly more $^{14}CO_2$ than did low humidity insects. Also, in the ^{14}C-palmitic acid study the low humidity starved insects released significantly more $^{14}CO_2$ than either low humidity control (FW) or dehydrated and starved (OO) insects. These data indicate that low humidity starved insects possibly rely more on lipid substrates, while glucose utilization in response to starvation appears to be more uniform.

In both of the radiolabelled studies, cockroach hemolymph contained small quantities of radiolabel at 15 d. Radiolabel associated with the hemolymph in the starved insects was not significantly different from either of the other two dietary regimens, in either of the radiolabel studies. However, in both radiolabelled studies the starved and desiccated insects' hemolymph radiolabel content was higher than that of both the control and

starved insects, but radiolabel values were too small to draw any valid conclusions from these results.

Examination of fat body tissue from the ^{14}C-glucose study indicated that significantly more radiolabel/mg of tissue was present in the low humidity starved (OW) insects than in the moderate/high humidity starved insects. In the ^{14}C-palmitic acid study, a large difference was noted in radiolabel content of fat body tissue in the starved (OW) insects in the 2 humidities, but no significant differences were associated with these values. These data, as others, suggest that when cockroaches are starved and under low humidity they retain more radiolabelled substrates originating from carbohydrate precursors and consistently utilize lipid reserves more heavily.

METABOLIC AND PHYSIOLOGICAL RESPONSES OF DEHYDRATED (FO) AND DEHYDRATED AND STARVED (OO) INSECTS

Dehydrated cockroaches (FO), when compared to control and starved insects, showed significantly higher terminal RQ and QO_2 values. RQ data similar to these have not been reported in the literature. Tucker (1977c) made reference to a RQ value of 0.76 for a few dehydrating adult, male cockroaches. Interpretation of RQ values with respect to substrate identification in dehydrated and dehydrated and starved insects is difficult and often is misleading.

Richards (1969) reported that cockroaches maintained under conditions of complete food and water deprivation had QO_2 values which decreased in a nonlinear manner and led to a general lowering of activity and/or basal metabolism. Results of the present study contradict the QO_2 data reported by Richards in that these insects showed a significant rise in QO_2 over the experimental period, but all individually-housed insects (after confinement) were noted to have reduced their physical activity. These results suggest that dehydrated insects significantly increase their metabolic rate, and that metabolic rate under these conditions may be independent of physical activity.

The dehydrated (FO) insects in both humidity levels had terminal body weights which reflected severe body water loss, and were significantly lower than initial body weights. These results agree with those of Wall (1970), Tucker (1977c) and Hyatt and Marshall (1985) in that dehydration reduces the total water content of the insect. Dry weight data suggest that cockroaches deprived of food and/or water utilized more body reserves than control insects.

Reduced food consumption rates observed in the dehydrated (FO) insects agrees with reports by others (Wall, 1970 and Tucker, 1977a) in that dehydrating cockroaches eat very little, if any. It appears that as

these insects dehydrate, water reserves (i.e. hemolymph and salivary reservoirs) are used to maintain water balance in the tissues. Dehydration of digestive tract tissues may have reduced food consumption rates, due to effects exerted by antidiuretic factors.

No radiolabel data are presented here on the dehydrated insects because in non-radiolabel, preliminary studies it was determined that results obtained from dehydrated (FO) insects are comparable to those obtained from dehydrated and starved (OO) insects. Therefore, in the radiolabel studies only the FW, OW and OO dietary regimens were examined.

Dehydrated and starved insects (OO) showed similar RQ, QO_2 and body weight change values to those of the dehydrated insects (FO). Since little or no food was consumed by dehydrated insects, it was anticipated that similar experimental results would be obtained for both dehydrated and dehydrated and starved insects. This was found to be the case.

No low humidity dehydrated and starved (OO) insects survived to 15 d in the ^{14}C-glucose study, therefore no comparisons can be made of these insects to the dehydrated and starved moderate/high humidity insects. The radiolabel content of the dehydrated and starved moderate/high humidity insects was equivalent to those recorded for the low humidity control (FW) and starved (OW) insects, and it

was significantly higher than those recorded in either the moderate/high humidity control or starved insects.

The 15 d whole body radiolabel content for dehydrated and starved (OO) insects in the ^{14}C-glucose study was slightly higher than that of the controls (FW), but less than that of the starved (OW) insects. Starved insects, able to maintain blood volume, may retain more carbohydrate stores while utilizing lipid reserves. In the ^{14}C-palmitic acid study the whole body radiolabel was higher in the dehydrated and starved (OO) insects than both of the other dietary regimens. These results suggest that the dehydrated and starved (OO) insects are not relying as heavily on lipid reserves as with the other dietary regimens.

Less fecal material was produced by dehydrated and starved (OO) insects than was produced by control insects in both humidity levels. This is consistent with the literature. Wall (1970) reported that fecal material excreted by dehydrated insects progressively becomes drier, until about 7 d at which time fecal pellets remain in the rectum for 2 - 4 d periods. Also, this fecal production was comparable to that of starved insects.

In the ^{14}C-palmitic acid study dehydrated and starved (OO) insect fecal radiolabel content was similar between the 2 humidity levels. This indicates that humidity played little, if any, role with respect to radiolabel

incorporation into feces. Obviously, water deprivation played the major role in concentrating radiolabelled materials in the fecal material.

The release of $^{14}CO_2$ by insects in the ^{14}C-glucose moderate/high humidity dehydrated and starved (OO) insects could not be compared to the low humidity dehydrated and starved insects due to the death of all the low humidity insects prior to 15 d. However, the moderate/high humidity dehydrated and starved insects in the ^{14}C-palmitic acid study released significantly more $^{14}CO_2$ than did low humidity dehydrated and starved insects. This indicates that dehydrated and starved insects in the higher humidity had $^{14}CO_2$ release values which were similar to the control and starved insects. Also, the low humidity dehydrated and starved insects in the ^{14}C-palmitic acid study released $^{14}CO_2$ quantities similar to the low humidity controls and less than the low humidity starved insects. This suggested that lipids may not have been the primary metabolic substrate of the low humidity dehydrated and starved insects.

Hemolymph radiolabel content of dehydrated and starved insects in the ^{14}C-palmitic acid study under both humidity levels were not significantly different. Again, no comparison can be made to the moderate/high humidity insects since no low humidity OO insects survived to 15 d in the ^{14}C-glucose study.

Radiolabel incorporation into fat body tissue in the ^{14}C- glucose study suggests higher content in the low humidity regimen, but no comparison to OO insects can be made. In the ^{14}C-palmitic acid study no significant difference was observed with respect to radiolabel incorporation into OO insect fat body tissue between the humidity levels.

Cuticular information obtained in these studies is far too inconclusive to interpret the results with confidence. Future cuticular studies should include methods which distinguish differences in cuticular radiolabel incorporation from radiolabel incorporation into associated cuticular materials.

SUMMARY

In general, insects in all 3 studies appeared to show little significant difference in the experimental parameters examined that could be attributed to variation in relative humidity. In part, this may be due to too small a range difference in imposed relative humidity regimens. Results support this hypothesis, and future research in this area should include wider humidity ranges to determine if, in fact, humidity does play a significant role in changing metabolic substrate utilization patterns.

However, some adjustments must have occurred, but they may not have been metabolic.

Without a doubt, dietary regimens were responsible for the majority of significant differences observed in these studies. Control, starved and dehydrated and starved insects in these studies reacted differently (physiologically and metabolically) to their experimental regimens. Many of the results obtained were confirmatory in nature, but are useful in comparisons of reports by other workers.

Cockroaches used in these studies did not show survival rates consistant with some reported in the literature, primarily those given by Willis and Lewis (1957). They reported that adult, male American cockroaches deprived of both food and water had survival rates (on average) of 40 - 45 days. The conditions under which these insects were held was not reported clearly, and may have varied greatly from those used in these studies. As reported, cockroaches used in these studies (designed to last 15 d) were individually caged and exposed to a continuous air stream. These experimental conditions may have had significant effects on the survival rates of these insects, especially those which were deprived of food and/or water.

In these studies, starved (OW) insects appeared to shift their metabolic substrate utilization patterns from

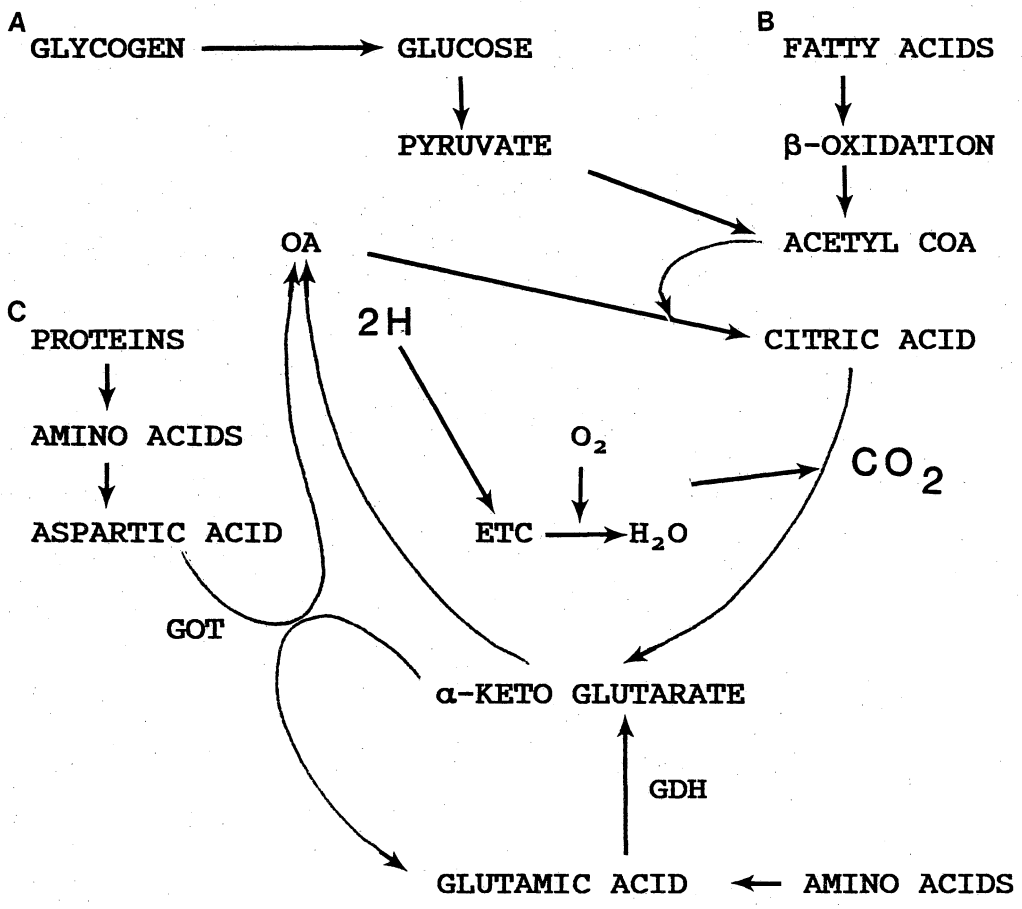
initial carbohydrate-based substrates to lipid-based substrates over the experimental period. This is supported by the respirometric and somewhat by the radiolabel studies. In the respirometric study, starved insects had terminal RQ values indicative of lipid metabolism, while QO_2 data are not representative of expected, increased oxygen consumption rates. Slightly increased radiolabel content of both the fat body tissue and whole bodies of the starved insects in the glucose study, compared to that of the control insects, implies that lipids may have been a metabolic substrate.

Both dehydrated and dehydrated and starved insects appeared to increase their metabolic rate (supported by the QO_2 data), but RQ data alone in these studies did not allow the determination of the primary metabolic substrate in question. Available data showed that increased fat body radiolabel in these insects, compared to that of the controls, could be the result of dehydrated fat body tissue which would show a higher radiolabel content/mg of tissue. The fact that dehydrated and starved (OO) insects essentially ceased the release of $^{14}CO_2$ from 1 - 2d in the glucose study indicated that these insects shifted their metabolic substrate utilization pattern from initial carbohydrate-based substrates to substrate(s) yet to be identified. The $^{14}CO_2$ data from the palmitic acid study suggest this shift was not directed specifically to lipids,

as these CO_2 data show no sharp fluctuations at this point comparable to the CO_2 data in the glucose study. In fact, in the radiolabelled palmitic acid study there was a reduction in radiolabelled CO_2 release at 2d compared to that at 4d. Future studies should be designed so that substrates metabolized by dehydrated and starved insects can be determined at specific intervals throughout this initial 2d period.

The results of both the respirometric and radiolabel studies indicated that starved and dehydrated and starved insects change their metabolic substrate utilization patterns in response to diet, and that humidity played a small part in this change. The starved (OW) insects appear to be the only group of cockroaches observed to change, as expected, to lipid-based metabolism when dietarily stressed. The dehydrated and starved (OO) insects (and possibly dehydrated (FO) insects used in the respirometric study) also appear to have changed their metabolic substrate utilization pattern, but biochemical identification of the specific substrate(s) has not been accomplished. Information in Figure 11 outlines possible metabolic pathways in insects utilizing carbohydrate-, lipid- or protein-based substrates. Insects which utilize primarily carbohydrates should have RQ values near 1.0, while those utilizing lipids have RQ values near 0.7 and those utlilizing protein have RQ values near 0.8. When

Figure 11 - Energy-producing biochemical pathways associated with the tricarboxylic acid cycle utilizing metabolic substrates produced by carbohydrate, lipid or protein catabolism.



[A] Carbohydrate catabolic pathway (RQ = 1.0)
[B] Lipid catabolic pathway (RQ = 0.7)
[C] Protein catabolic pathway (RQ = 0.8)

Note - American cockroaches deprived of both food and water showed RQ values which reflected catabolic patterns characteristic of multiple substrate utilization (refer to Section 4.1).

amino acids such as aspartic acid and glutamic acid are transaminated to oxaloacetate and alpha ketogluterate, respectively, these substrates enter the tricarboxylic acid cycle as shown in Figure 11, and result in RQ values resembling those obtained during carbohydrate-based metabolism. In view of the respiratory quotient and oxygen consumption data, it may be suggested that dehydrated and dehydrated and starved insects were metabolizing primarily lipid and amino acid substrates during the latter portions of their 15 day experimental periods.

The American cockroach originally evolved in the tropical regions of the world, and has adapted to many different environmental conditions so well that it has become an ubiquitous urban pest. Frequently, this insect is confronted with periods of water and/or food deprivation, and metabolically has adapted by relying on stored metabolic reserves, or by reducing its physical activity to conserve metabolic reserves, until water and/or food become available. From this study, it is obvious that the absence of water from the insect's environment is a major limiting factor associated with this insect's overall survival and success.

In conclusion, it appears that the American cockroach has evolved a series of complex metabolic and behavioral adaptations that enable it to physiologically compensate for periods of dehydration and/or starvation. When these

insects are deprived of food, they rely on metabolic reserves principally from the fat body which are distributed to tissues by the circulating hemolymph. When deprived of water and/or food, the hemolymph volume dwindles and these insects apparently rely on protein-based metabolic reserves. These reserves either have been transported to the dehydrating tissues by the dwindling hemolymph (prior to substantial hemolymph volume reduction), or naturally occur in the cells and may be biochemically converted to substrates required to provide energy and some metabolic water under these conditions.

Further research still is needed in the area of primary catabolic substrate identification in relation to specific changes in metabolic substrate utilization patterns. More emphasis must be placed on identification of these substrates in order to further our basic understanding of general insect metabolism.

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