PHARAOH ANT CONSUMPTION OF FLUIDS USED IN HOSPITAL ENVIRONMENTS

By

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I dedicate this thesis to God, The Risen Lord and Creator of all things.

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Monomorium pharaonis (L.), the Pharaoh ant, is a pest species which inhabits buildings throughout the world. Its populations are difficult to control. When in hospitals and other health-care environments, these ants are often found nesting in places as diverse as operating rooms, linen closets, baby incubators, and inside unopened packages of intravenous tubing. Pharaoh ants have been observed feeding upon human secretions and on intact epidermis. In addition they have been reported to consume patient-care fluids, such as dextrose and saline solutions.

Small Pharaoh ant colonies that were starved for 2 d consumed significant amounts of either blood, 5% dextrose, Ensure® dietary supplement, 0.9% NaCl solution (saline), or plasma when given 24 h access to each fluid separately. Comparison of consumptions based on these no-choice exposures indicated that significantly more 5% dextrose was consumed among all the other fluids. Simultaneous, or choice, exposures of

the same fluids again showed the most significant consumption occurred on 5% dextrose. Counts of feeding Pharaoh ants, which were starved for 3 d and given individual access to the fluids dried on hospital linens, were highest on the Ensure® dietary supplement, followed by the 5% dextrose.

CHAPTER 1
INTRODUCTION

Monomorium pharaonis (L.), commonly known as the Pharaoh ant, has a close association with human habitats (Wheeler 1910), and is probably one of the world's most studied ants. It is a tropical ant that needs both high humidity and high temperatures (Hölldobler and Wilson 1990). In temperate zones, it lives in buildings, such as hospitals, which are heated and have indoor plumbing throughout the structure (Edwards and Short 1990). Beatson (1972) reported that Pharaoh ants can carry several pathogens, including *Streptococcus*, *Staphylococcus*, *Escherichia coli*, *Bacillus*, *Pseudomonas*, and others. This discovery sparked a brief interest in the pathogenic vector capacity of Pharaoh ants that lasted into the 1980's. More recently, independent observations by Edwards and Short (1990) and (Edwards and Short 1990, Eichler 1990) of Pharaoh ants feeding on human patients rekindled interest in this species. Additionally, a limited number of tramp ant species, including Pharaoh ants, have been implicated as vectors of hospital-acquired diseases (Fowler 1990), and were associated with a higher incidence of nosocomial infections (Richards et al. 1999). The ability of Pharaoh ants to move between patients receiving medical treatment and hospital areas that may harbor pathogens, such as sewage, plumbing, food-preparation areas, and even diseased patients suggests that Pharaoh ants have the potential of spreading pathogenic diseases throughout a hospital. Eichler (1990) considered Pharaoh ants to be one of the most dangerous insects in health-care environments.

The disease vectoring potential of Pharaoh ants, like that of other arthropods more traditionally associated with disease transmission, such as cockroaches and flies, may be directly linked to their food preferences and foraging behavior. Therefore, Pharaoh ant consumption of liquid substances that are specifically used in patient care needed to be examined more closely. The objective of this research was to determine which fluid(s) used in patient care attract Pharaoh ants. Two experiments were conducted to determine if Pharaoh ants consume (1) liquid fluids used in hospitals (Chapter 3), and (2) dehydrated hospital fluids (Chapter 5). A third experiment (Chapter 4) compared the attraction among the liquid hospital fluids when presented simultaneously to Pharaoh ants.

CHAPTER 2
LITERATURE REVIEW

General Ants

There are over 9,500 known species of ants in the world (Bolton 1995). Ants are eusocial insects which live in colonies comprised of multiple castes, usually morphological, but sometimes temporal (Oster and Wilson 1978), and multiple developmental stages. Eusocial behavior in ants is seen in the division of labor/function amongst different castes, in the presence of overlapping generations, and in the tending of immature brood by non-parental adults (Hölldobler and Wilson 1990)

Tramp Ants

Ants which are harmful to human activity are classified as pests, and are often considered to be tramp species. Tramp species have many traits that enabled them to be synanthropic. They are attracted to changing environments that are linked strongly to human activity (Passera 1994), and often nest inside buildings (Ebeling 1978). Many have been transported to new countries around the world via human commerce (Riley 1889). Tramp species tend to migrate quickly in response to changes in environmental conditions or food availability, or to overpopulated colony size (Passera 1994). Many are polydomous colonies that lack aggression between neighboring nests, so that workers, queens and brood are freely exchanged between nests (Passera 1994). Within each nest, tramp species are often polygyne, that is, they have many fertilized queens (Ebeling 1978, Passera 1994). Sexual castes often engage in intranidal mating rather than having nuptial flights, even when wings are present (Hölldobler and Wilson 1990). Colony

reproduction takes place by sociotomy, or budding, when workers and newly mated queens leave to start a new nest nearby (Passera 1994). Tramp ants are usually monomorphic, with only one morphological worker caste, and are always very small (Passera 1994).

Pharaoh Ant

Monomorium pharaonis (L.) (Formicidae: Myrmicinae), the Pharaoh ant, is a worldwide pest (Wheeler 1910, Hölldobler and Wilson 1990) that is closely associated with humans and is considered a tramp ant (Passera 1994). Pharaoh ant colonies exhibit a temporal division of labor (Sudd 1957b) in which younger workers spend most of their time tending the brood and queens while older workers primarily forage. In the absence of one caste, members of another caste will perform the additional tasks. Pharaoh ants feed on a wide variety of foods (Bellevoye 1889, Peacock et al. 1950, Sudd 1960, Granovsky and Howell 1983, Edwards 1986, Edwards and Abraham 1990). They also feed on humans, where they have been observed recruiting to body and wound secretions, (Beatson 1972, Edwards and Baker 1981a, Granovsky and Howell 1983, Edwards and Short 1990, Eichler 1990). In addition, they have been observed recruiting to or feeding on the unbroken skin of babies' cheeks, lips, neck folds and backs of ears has been reported (Steinbrink 1978, Eichler 1990). Pharaoh ants are especially well-adapted to taking advantage of any available space for nesting sites, and are primarily found nesting inside walls and crevices (Wheeler 1910). However, colonies have been discovered in even more unusual locations, such as in linens, between sheets of paper (Ebeling 1978), in intravenous tubing packages, in surgical and resuscitation equipment (Beatson 1973), and in baby incubators (Steinbrink 1978, Eichler 1990).

Origin and Distribution

The Pharaoh ant was originally designated as *Formica pharaonis* Linneaus 1758, and had six different names until it was eventually named *Monomorium pharaonis* (L.) (Bolton 1994). The Pharaoh ant's origin is uncertain, with many researchers contending that the ant originated in the tropical regions of Africa (Ebeling 1978). However, Emery (1922), Wilson and Taylor (1967) and Bolton (1987) agreed that India is the probable origin of the Pharaoh ant. (Arnold 1916) stated South America was the original location of this ant. The Pharaoh ant is common and widely prevalent in all these areas, and is easily spread by human commerce. Hence, its true origin may never be determined.

The Pharaoh ant is a tramp species that is strongly associated with humans (Edwards 1991), and was proclaimed as the "most widely distributed of all ant pests" by Edwards and Short (1990). They listed its documented presence to the United States, Canada, South America, Egypt, North Africa, Europe, Russia, Australia, and Japan. The Pharaoh ant is also present in the Hawaiian Islands (Reimer et al. 1990).

Biology

Pharaoh ant workers are monomorphic, and are very small, with a length of 1.5-2.0 mm. They are reddish to orange-brown in color, have a two-segmented petiole with each segment having a node, and their gasters have a white stripe on the anterior portion of its dorsal surface (Haack 1987, Harris 1991) Each antenna has 12 segments, with the final 3 segments forming a visible club. Adult males are winged, black in color, and slightly larger than the workers, but smaller than queens. In natural colonies, males appear infrequently, although male eggs are laid continuously (Peacock et al. 1954). At 4 mm in length, queens are the largest caste of the Pharaoh ant. Virgin queens can be

distinguished from egg-laying queens by the presence of their wings, which are bitten off after mating.

Reproductive males and females emerge in the nest with wings, but they do not have nuptial flights. Instead, they mate within the nest (Peacock and Baxter 1950). Wing muscles in the thorax of mated queens hystolize so that the thorax is modified to contain a thoracic crop that is supplemental to the gaster's crop (Caetano 1990).

Within the nest, brood is normally separated into distinct piles that are sorted by age (Peacock and Baxter 1949). Pharaoh ant eggs are round and stick together (Harris 1991). Larvae are immobile, and must be transported and cared for by adult workers. Sexual brood develops faster than worker brood, but differentiation between the two brood castes is difficult until the gut color of worker larvae changes to black, or the sexual larvae becomes noticeably larger than the worker larvae (Peacock and Baxter 1950).

Lifespans of Pharaoh ants differ among the castes. Workers live ~9-10 weeks, males normally live ~3 weeks, and queens live ~39 weeks, (Peacock and Baxter 1950), although they can live as long as 52 weeks (Edwards 1987).Pharaoh ant colonies can vary tremendously in size (Peacock et al. 1955a) and can contain thousands of individuals, including many fertile queens. Queen fecundity is directly related to the number of last instar larvae present in the colony (Börgesen and Jensen 1995). Natural Pharaoh ant colonies tend to have a 1:1:1 ratio of workers:eggs:larvae (Peacock et al. 1955b). Pharaoh ant colonies are polydomous, and exchange queens, workers and brood among nests (Edwards 1991). Migrating groups of ants often form a temporary nest while searching for a more permanent nest site (Edwards and Baker 1981a). New nests are

formed via budding (Hedges 1997), since Pharaoh ants do not have nuptial flights (Ebeling 1978).

Peacock and Baxter (1949) originally stated that Pharaoh ants preferred temperatures ~27°C and 80% RH. Harris (1991) demonstrated that Pharaoh ants preferred a lower relative humidity of ~65%. In temperate regions, Pharaoh ants nest primarily indoors (Ebeling 1978).

Foraging and Food

Pharaoh ants engage in extensive foraging, and are most active at night {Hedges, 1997 #721}. They likely use "guideline orientation" (Klotz and Reid 1992) to follow edges and cracks of structures to find food sources. They will also forage via ducts and wall spaces aluminum window and door frames, and they can forage up to distances of 45 m (Vail and Williams 1994).

Pharaoh ants are well-known for their consumption of a wide variety of foods (Smith 1965, Edwards and Baker 1981a). Some researchers contend that the Pharaoh ant is primarily attracted to sugars (Granovsky and Howell 1983), while others think that they primarily consume proteins (Edwards and Abraham 1990). Other research indicated that Pharaoh ant colonies compensated for what was lacking nutritionally, whereby laboratory colonies that were reared on carbohydrates exhibited more interest in protein baits, and vice-versa (Edwards and Abraham 1990). Thus, the food preference of Pharaoh ants is variable and dynamic, changing with nutritional needs (Hölldobler and Wilson 1990), and time (Edwards 1986).

Medical Importance

The Pharaoh ant is a tropical ant and needs high humidity and high temperatures to survive (Peacock and Baxter 1950, Peacock et al. 1955c). In temperate zones, it is

dependent upon heated buildings, and is a common pest species found in hospitals and other health care facilities world-wide. Although hospital infestations of Pharaoh ants were already recognized as an inconvenience, the discovery of several pathogenic organisms, including *Streptococcus* spp*., Staphylococcus* spp*., Escherichia coli, Bacillus* spp*., Psuedomonas* spp*., Klebsiella* spp., and *Clostridium* spp., on hospital populations of Pharaoh ants (Beatson 1972), created interest in the potential for Pharaoh ants to transmit diseases to humans.

In Brazil, the potential for Pharaoh ants to vector hospital-acquired (nosocomial) infections may have been especially high, as this country had some of the highest rates of intra-hospital infections. In these hospitals, it was determined that only tramp ant species, including Pharaoh ants, were near patients (Bueno and Fowler 1994). Besides being potential vectors, Pharaoh ants can cause skin irritations and lesions (Eichler 1990). Their small size enables them to crawl through gauze covering bandaged wounds and ants as small as 2.0 mm in length can cause intense pain when foragers walk on open wounds (Eichler 1990). Pharaoh ants also have been found feeding directly on open wounds of burn-unit patients (Anon. 1986), on or under bandages covering wounds (Cartwright and Clifford 1973), on premature newborns in incubators (Edwards and Short 1990), on bedridden elderly or postoperative patients (especially patients who were leaking body fluids)(Anon. 1974), and in intravenous tubes that supply fluids to patients (Beatson 1973, Edwards and Baker 1981b).

Control

Once ensconced in a structure, Pharaoh ant populations are extremely difficult to eradicate partially due to cryptic habits, including a tendency to live inside walls and under foundations. It is often difficult to locate Pharaoh ant nests without damaging the

building's structure (Vail 1996). Many different methods of Pharaoh ant elimination have been attempted, with varying degrees of success. The efficacy of pesticide sprays and dusts, stomach poisons, baits containing toxicants, and, baits containing insect growth regulators (IGRs) have all been investigated.

In the 1990s, sprays and dusts were usually ineffective against Pharaoh ants, because they affected only a small percentage of workers (Williams 1990) due to the fact that less than 25% of workers forage and many of the chemicals were in the pyrethroid class and highly repellent. Their use was even hypothesized to result in budding (Williams and Vail 1994), since Pharaoh ant colonies respond rapidly to disturbance (Peacock et al. 1955b, Passera 1994). Eichler (1990) believed that insecticide spraying of Pharaoh ant infestations to be a form of malpractice. In addition, the effectiveness of sprays was believed to have been reduced if multiple nests are present in the general area (Williams 1990).

Stomach poisons work too quickly and reduce worker numbers so much that poisons are not distributed to queens and brood (Edwards 1975). Colonies can be perpetuated by as few as 100 workers and 50 brood (Peacock et al. 1955a) or even with as few as 5 workers, 30 eggs, 19 larvae, and 3 pupae (Vail 1996). Therefore, to eliminate Pharaoh ant populations, it is essential for toxicants to reach the queens and brood.. Baits should be comprised of a slow acting, non-repellent toxicant incorporated into an attractive bait matrix so that the bait is consumed and spread through the colony via trophallaxis. Eichler (1990) recommended meat-based bait matrices, and sodium arsenate or chlordecone. Finally, he recommended pre-baiting to determine what food attracts the Pharaoh ants at the time, since they are known to change food preferences, he

suggested placing baits near locations that they would likely forage, such as sinks. He also suggested denying ants access to alternative food sources, so that the bait would be their only food source.

Klotz et al. (1996) noted that boric acids were used against ants as early as the late 1800s (Riley 1889), and are still important in ant control. They cited a recommendation by Wright and Stout (1978) for 2% boric acid in either a liquid or solid bait. Klotz et al. (1996) tested the efficacy of 1% boric acid in sucrose solution and 0.9% hydramethylnon with both limited and continuous availability in the laboratory. They determined that colony growth was reduced, but not eliminated, after 3 d exposure to boric acid bait. In contrast, continuous feeding of boric acid bait resulted in elimination of all colonies. Colonies exposed to hydramethylnon had 90% reduction of workers by 3 wk and 90% reduction of brood by 4 wk; queens were eliminated in 2 wk. They concluded that boric acid-sucrose solutions might provide an alternative to other commercially available Pharaoh ant baits.

Many toxicants such as Mirex are no longer used due to carcinogenicity (Eichler 1990). Insect growth regulators (IGRs) which mimic juvenile hormones, have been developed (Nation 2003). IGRs often contain either the active ingredient methoprene or pyriproxyfen. Insect growth regulators are slower-acting than stomach poisons because they affect only queen and brood: they induce sterility in queen and disrupt brood development (Edwards 1975). The workers remain active for several weeks, but are not able to start new colonies due to death of queens and brood.

Oi et al. (1996) compared the efficacy of several different applications to determine if indoor Pharaoh ant infestations could be controlled by exterior treatments,

since Pharaoh ants have been observed foraging on exterior wall surfaces and outside buildings (Haack 1991, Oi et al. 1994, Vail and Williams 1994). They tested several methods registered for ant control: a contact insecticide (cyfluthrin), a granular contact insecticide (diazinon), a granular bait containing a contact insecticide (propoxur), and a granular bait containing a delayed-action toxicant (hydramethylnon). They determined that exterior application of the delayed-action toxicant hydramethylnon resulted in successful control indoor Pharaoh ant infestations, while all other tested treatments resulted in increased foraging. They concluded that exterior baiting could reduce the need for indoor pesticide applications. In 2003, a new compound, fipronil (Termidor, BASF, Research Triangle, NC) received registration for ant applications. It was an old idea of spraying the perimeter of a building for ant control with a new compound that had important bait-like characteristics. Fipronil is a non-repellent, slow acting toxicant. Successful control of Pharaoh ants foraging outside and inside homes treated on the perimeter only with fipronil, has been demonstrated (F. Oi and J. Jonovich, personal communication) according to label directions (25 cm up and 25 cm out).

Vail et al. (1996) evaluated the efficacy of different bait formulations of the IGR pyriproxyfen as an exterior perimeter treatment against Pharaoh ant infestations. They noted that while pyriproxyfen had already been evaluated as an effective control agent against most urban insects tested previously, and despite the demonstration of its effectiveness against Pharaoh ants in the laboratory (Vail and Williams 1995), no IGRs were commercially available for use against Pharaoh ants. They determined that the baits formulated with various amounts of pyriproxyfen in two different bait matrices were all successful in controlling the ant populations, although complete elimination did not occur

until 16 wk after treatment. They observed that brood rapidly declined in treated colonies. Dyes used in bait formulations was visible in larvae, queens and workers, indicating thorough distribution of baits throughout the colonies. Finally, they concluded that application of an IGR bait 1 wk before application of a metabolic inhibitor might provide both quick mortality and long-term control, and they stated that vertical placement of baits aimed at Pharaoh ants might prevent other ant species from consuming baits.

Oi et al. (2000) investigated the distribution of baits containing the IGR, pyriproxyfen, and the metabolic inhibitor, hydramethylnon, among multiple Pharaoh ant colonies. They determined that the IGR did not kill adult worker ants quickly so that more worker ants lived long enough to distribute the bait to all nest sites. In contrast, hydramethylnon caused rapid mortality in adult ants, and ants from nearby nests migrated into sites whose populations declined due to metabolic inhibitor mortality. They further determined that the IGR pyriproxyfen resulted in a slow, gradual decline of both workers and queens in all nest sites, and lasted a long time, in contrast to the metabolic inhibitor hydramethylnon, which worked more quickly, but did not last as long. Finally, they postulated that longer survivorship of workers increased the amount of trophallaxis between ants of different nest sites and increased the distribution of the IGR, pyriproxyfen, among multiple nests, thereby reducing the possibility of re-infestation. They concluded that the mode of action and speed of action of bait toxicants should be evaluated when selecting ant baits for polydomous colonies such as the Pharaoh ant.

CHAPTER 3
NO-CHOICE LIQUID TEST

Introduction

Pharaoh ants' consumption of a wide variety of substances (Bellevoye 1889, Granovsky and Howell 1983, Edwards and Abraham 1990) and their abundant presence in hospitals is well-documented (Green et al. 1954, Beatson 1972, Edwards and Baker 1981b). Although Pharaoh ants are primarily sampled in kitchens and bathrooms because it is assumed that there will be an abundance of water and food available to sustain ant colonies, Vail and Williams (1994) found that they are capable of foraging up to 45 m.

Pharaoh ants have been found in hospitals and health-care facilities (Eichler 1990, Fowler 1990). They have been discovered inside a permanently installed tracheotomy tube and intravenous tubes (Weidner 1982). Pharaoh ants have been observed feeding upon patients within different hospital areas. In neonatal units where newborns are placed inside special incubating units, they have been observed feeding on intact skin of newborn infants such as cheeks, lips, neck folds and backs of ears (Steinbrink 1978) as cited in (Eichler 1990). They have been observed feeding on burn patient open wounds (Weidner 1982). Their small size enables them to crawl through gauze; thus, they have also been found under bandages of covered wounds (Beatson 1972). Finally, Pharaoh ant infestations have been located in beds of bedridden elderly or postoperative patients (especially patients who were leaking body fluids) (Anon. 1974).

Pharaoh ants can also be associated with other fluids present in a hospital environment. Suppurating wounds exude plasma, which is the non-cellular portion of

whole blood. Additional fluids can also be present, such as saline or dextrose solutions that are administered either intravenously or topically (AABB 2002, Wenzel et al. 2002). Both types of administration of saline and dextrose decrease moisture loss in the absence of epithelial tissue and in situations of limited fluid consumption (AABB 2002); topical use also will sterilize wounds and decrease the potential for infection at injury site. Pharaoh ants might be attracted to these fluids or to substances present in patient blood or plasma. In addition to saline and dextrose intravenous and topical administrations, many patients also receive liquid dietary supplements after surgery, via tubing that is inserted into patient's stomach (Wenzel et al. 2002). These fluids provide another potentially attractive food source to Pharaoh ants. Dextrose, saline and liquid dietary supplements are administered directly into patient via tubing. Anecdotal reports from nurses (S. Cobb, personal communication) indicate that medical practices such as tube clearing (i.e., procedure of squirting liquid out of tube prior to insertion of tube into patient) often result in placement of liquids on or near the patient bed. Thus, each of these liquids is available not only at insertion point in patient's body, but might also be available in abundance throughout a hospital room, particularly close to the patient's bed.

Pharaoh ants occur in hospital rooms and have been documented to feed upon patients, but no previous study has been undertaken to determine consumption of patient-care fluids and human fluids. Even though ants were documented as feeding on wounds, no examination has investigated consumption of human body fluids. Thus, my objective was to determine if Pharaoh ants consume fluids used specifically for patient-care in hospitals.

Materials and Methods

Ants

Pharaoh ant test colonies were extracted from large colonies housed and reared at the Urban Entomology Laboratory, University of Florida, Gainesville, FL. Rearing temperatures ranged from 21.9°C – 29.4°C with relative humidity between 20-53%. (Appendix A).

Colonies consisted of 250 workers, 3 dealate queens and approximately 50 mg brood. Half of the workers were selected from the inside of nesting cells to ensure the presence of nurses and 125 workers were selected from outside of the nesting cells, at foraging locations such as exterior surface of water vials, to ensure the presence of foragers (Haack 1987, Haack and Vinson 1990, Haack et al. 1995). Brood included eggs, all larval stages, and pupae. See Appendix B for full details on selection of ants and preparation of experimental test colonies.

Foraging Arena

Foraging arenas contained one nesting cell, one water vial, and one microcapillary tube (Fig. 3-1). Foraging arenas consisted of clear plastic boxes (27 by 19 by 10 cm deep; Pioneer Plastics, Dixon, KY) which were coated with Fluon® (Asahi Glass Fluoropolymers, Downington, PA) on interior sides to prevent ant escape. Nesting cells consisted of sterile, covered plastic Petri dishes (50 mm diameter by 15 mm deep; Falcon® Model No. 351007, Becton Dickinson and Co., Franklin Lakes, NJ) filled halfway with Castone dental plaster (Model No. 99045, Dentsply International, York PA). A circular indentation was made in plaster by inserting the cap of a 15 ml vial (Model No. 55-9, Thornton Plastics, Salt Lake City, UT) into freshly mixed plaster in the

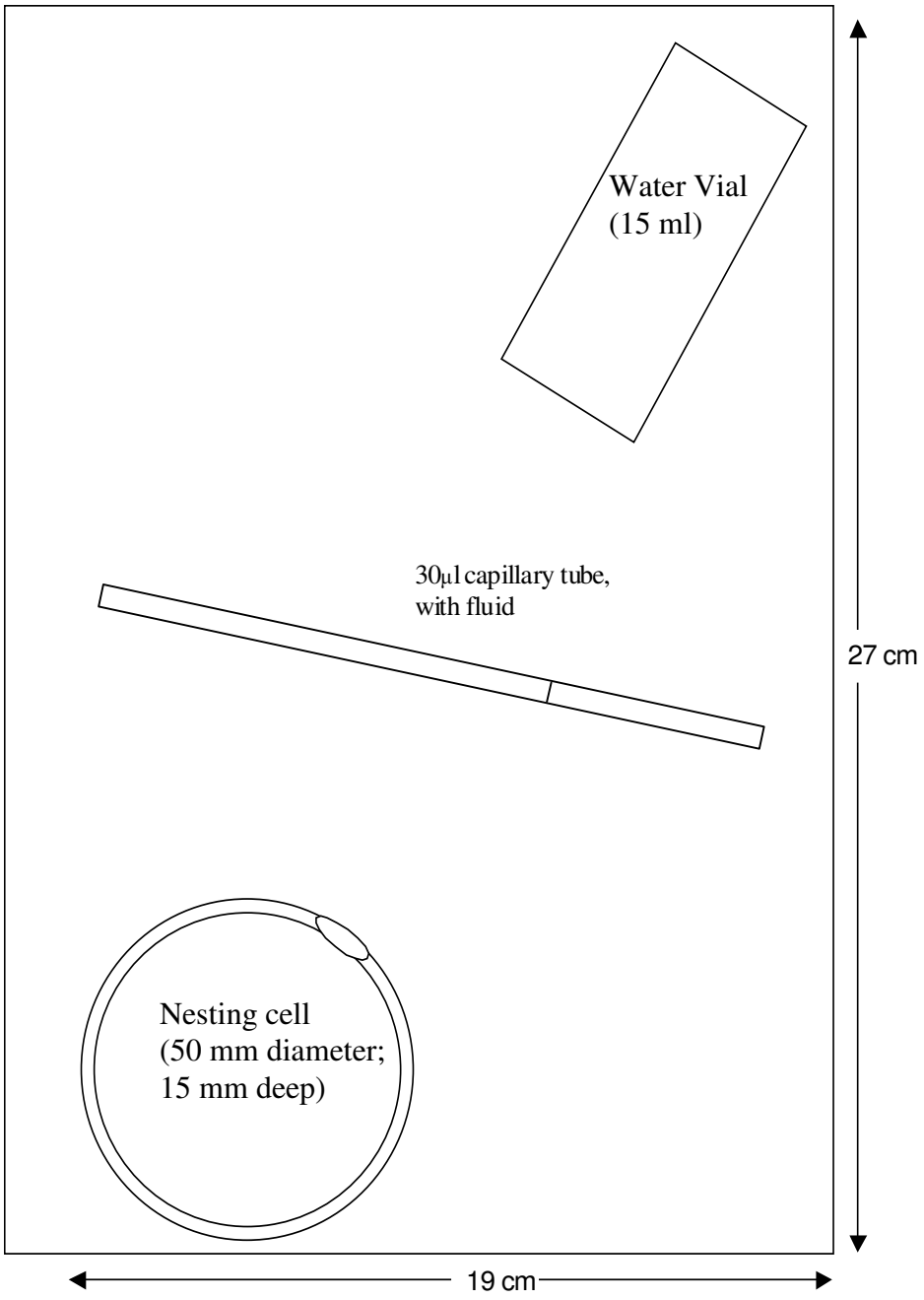

Figure 3-1. No-choice liquid arena with water vial and nesting cell placed in diagonally opposite corners of test arena. A microcapillary tube containing one fluid was placed in the center of arena. Both test arenas (with ants) and control arenas (no ants) were placed adjacent to each other to comprise test pair. Test arenas contained 125 inside ants, 125 outside ants, 3 queens and ~50 mg brood (including pupae, eggs and all stages of larvae). Control arenas lacked ants.

base of a Petri dish. The vial cap was removed after plaster was thoroughly dried (24 h) and hardened, leaving an indentation (33 mm diameter by 2 mm deep) and rough sloping edge (Peacock and Baxter 1949) for ants to rear brood. One entrance hole (~5 mm diameter) was melted into the upper edge of the Petri dish base via insertion of the metal tip of a hot glue gun (*sans* glue) just above level of hardened plaster. Immediately prior to placing ants in arenas, Petri dish bases were immersed in a container of tap water for 30 min to saturate the plaster. Saturated bases were removed from water and excess water was blotted from all surfaces. A Petri dish lid was placed on top of the base and the entire nesting cell was placed into the foraging arena with the entrance hole facing the opposite diagonal corner of the arena.

Water was provided *ad libitum* in a 15 ml vial (Model No. 55-9, Thornton Plastics, Salt Lake City, UT) with three large cotton balls inserted into the top to prevent leakage and to provide easy access for ants. A water vial was placed in the opposite diagonal corner of the arena across from the nesting cell (Fig. 3-1). One test arena containing ants and one control arena without ants were placed adjacent to each other and comprised a paired experimental unit for each replication. Control arena data were used to quantify and adjust for evaporation of fluids. All arenas were covered with clear plastic lids to maintain humidity and temperature. Airflow was maintained in the room by the building's ventilation system during test period.

Experimental Fluids

Five fluids commonly used in hospital environments for patient-care were used in this no-choice test. Test fluids can be provided to patients via insertion of tubing, either intravenously (i.e., injected directly into bloodstream) or into intraperitoneally (i.e., into

stomach). Fluids consisted of human blood cells, human plasma, saline (0.9% sodium chloride); 5% dextrose (D-glucose), and Ensure® (Vanilla Plus, Ross Products Division, Abbott Laboratories, Columbus, OH).

Blood cells and plasma (all other blood components except blood cells) were obtained by centrifugal separation of donated whole blood, and were tested as individual fluids since these two major constituents of whole blood differ greatly in composition and structure. Blood cells can break down within 5 to 7 d (AABB 2002) without addition of anti-coagulants due to enzymatic activity of intracellular blood proteins and cell death. Whole blood samples contained heparin anticoagulant, which prevents clotting and increases shelf-life of blood cells to ~42 d. Plasma and blood cells were stored together and shared a shelf-life of ~42 d, which is typical for whole blood used in trauma and emergency situations (Memmler et al. 1996, AABB 2002). Whole blood was obtained on its expiration date after 30 d in refrigerated storage at LifeSouth Blood Bank (Gainesville, FL). Therefore, all whole blood was aged 30 d and contained heparin anti-coagulant.

Whole blood was centrifuged to separate blood cells (bottom layer) from plasma (top layer). Plasma was pipetted out and placed in separate test tubes. Plastic caps were inserted into test tube openings to prevent leakage. Blood and plasma samples were placed into a resealable plastic bag (0.95 L, Ziploc®, S.C. Johnson & Son, Inc., Racine, WI) with two paper towels folded inside to absorb fluid in case of spillage. Resealable plastic bags were placed inside a thick-walled Styrofoam cooler. Ice (in separate resealable plastic bag) was present in the cooler to keep blood cells and plasma samples cool during transport. Blood cells and plasma were refrigerated and used within one week of acquisition. Blood cells and plasma were both used with appropriate biohazard

procedures. Shands Hospital Burn Unit provided sealed, unopened bags of 5% dextrose and 0.9% NaCl. Dextrose and saline were placed and maintained in a refrigerator (along with blood cells and plasma) at ~4.1°C before use. Dextrose and saline portions were squirted into dry, clean test tubes (150 mm by 10 mm inner diameter, No. 9800, Pyrex Labware, Corning Inc., Corning, NY) for use in experiments. Bags were sealed immediately after use and remained out of the refrigerator for no longer than 5 min while removing fluid for testing.

Ensure® (Ensure Plus® Vanilla), a commonly used flavor in hospital environments (S. Cobb, personal communication), was used in experiments because Pharaoh ants have been observed feeding upon dietary supplements in hospitals and other health-care environments by pest management personnel (W. Wright, Jacksonville PMC Seminar, personal communication). Ensure® is a dietary supplement that contains substantial proportions of proteins (26%), sugars (17%), and lipids (17%).

Experimental Design

The experiment was designed as a randomized complete block, blocking on colony. Each block consisted of Pharaoh ant colonies (125 inside workers, 125 outside workers, 3 dealate queens and ~50 mg brood) obtained from the same parent colony and randomly assigned to each treatment (fluid type). Ants were selected from nine different parent colonies. A single fluid was offered to each test colony to determine the consumption and preference of fluids under no-choice exposure. One test arena and one control arena were located adjacent to each other. There were 17 replications for a total of 85 test arenas and 85 control arenas. Fluids were tested over a six week period from

January 24, 2003 to March 10, 2003; room temperature and humidity were between 25.7 $\pm$ 3.7°C and 20-53%, respectively.

The temperature and relative humidity of test arenas and control arenas, in contrast to room temperature and humidity, was determined in a separate study on one day (June 2003) when room temperature was 26.4°C and room humidity was 38%. This was accomplished by placing a digital thermometer and hygrometer inside each of 13 paired test and control arenas for 2 min, a length of time sufficient for readings to stabilize but not long enough for ants to invade electronic equipment. Colonies inside test arenas were identical in composition to those used in this no-choice consumption test.

Water consumption was determined in a separate study over a three week period in November, 2003 during which room temperature and humidity were maintained between 28.1 $\pm$ 1.4°C and 43-48%. Ants were provided with water in microcapillary tubes (30 µl volume, 95 mm by 10 µm inner diameter; Model No. 21-380-9C, Fisher Scientific, Atlanta, GA) in the same manner that test fluids were provided. Water control tests were replicated using the same 9 colonies in the same sequence used for test fluids with a total of 17 test arenas and 17 control arenas.

Pharaoh ant colonies were starved for 48 h before placement of fluids into arenas; water was available *ad libitum* in all arenas, including water controls, via a 15 ml cotton-stoppered vial during starvation period (Haack and Vinson 1990, Haack et al. 1995). After 48 h of starvation, a microcapillary tube containing one test fluid was added to each arena. Ants were allowed to feed for 24 h. Then, fluid loss was measured gravimetrically in each arena.

Fluid Measurements

Each empty 30 μL (95 mm long) microcapillary tube was weighed via a digital scale to $\pm$0.1 mg, and the mass of the empty microcapillary tube was recorded. Each tube came pre-marked with a green fill line located 67 mm from tip of tube. Each microcapillary tube was filled to the 67 mm mark and held ~21.2 μL of test fluid. Mass of the microcapillary tube with fluid was weighed and recorded. The amount of fluid provided to all control and test arenas was calculated for each arena by subtracting the mass of the empty microcapillary tube from the mass of the tube containing initial mass of provided fluid (Wei and Johnson 1996). Thus, initial provided amounts of each fluid were obtained for control arenas and for test arenas.

In addition to initial weights of food provided to arenas, data collected during the experiment included post-evaporation weights of fluid-loaded tubes for control arenas. Final, post-evaporation weights of control arena fluids were obtained by subtraction of the appropriate empty microcapillary tube mass (Wei and Johnson 1996) from the initial weight of the microcapillary tube loaded with food.

Finally, data collected during the experiment included post-consumption weights of fluid-loaded tubes for test arenas. Final, post-consumption weights of test arena fluids were obtained by subtraction of the appropriate empty microcapillary tube mass (Wei and Johnson 1996).

Fluid loss in control arenas was calculated by subtracting the amount of fluid initially provided from the amount of fluid remaining; in control arenas, fluid loss was due entirely to evaporation. Fluid loss in test arenas was comprised of both consumption and evaporation; therefore, consumed quantities were determined by adjusting for

evaporation. Provision of fluids via microcapillary tubes enabled fluids to retain constant surface areas, and greatly minimize evaporation. Although several consumption formulas were considered (Appendix C), because it is a standard method of measuring consumption in nutritional ecology literature, a slightly modified version of Waldbauer's (1968) formula was used to correct for evaporation rates of both initial and final fluid quantities in test and control arenas as follows:

$$C = (1 - \tfrac{1}{2}[Y/G]) * (W - [L + (Y/H)L])$$

where

C = corrected consumption,

W = mass of fluid provided (g) in test arena,

L = mass of uneaten fluid and ants remaining in fluid (g) in test arena,

G = mass of fluid provided (g) in control arena,

H = mass of fluid remaining (g) in control arena,

Y = G – H = weight loss of control fluid,

Y/G = weight loss of control fluid / initial mass of control fluid (= a, in Waldbauer 1968),

Y/H = weight loss of control fluid / final mass of control fluid (= b, in Waldbauer 1968).

Statistical Analysis

Evaporation of patient-care fluids in control arenas was analyzed via a one-tailed Student's *t*-test ($P = 0.05$; SAS Institute 2000) to determine if the amount of evaporation was significantly different than zero (H_o: $\mu = 0$) (Fig. 3-2). Evaporation of each fluid was also compared via analysis of variance, and means were separated using Tukey's HSD ($P = 0.05$). In a separate study, means of humidity and temperature inside control arenas

were compared via analysis of variance; means were separated using Tukey's HSD (P = 0.05; SAS Institute 2000).

Negative consumption values (9 of 101 values) were truncated to zero. Consumption of patient-care fluids and water was analyzed via a one-tailed Student's t-test (P = 0.05; SAS Institute 2000) to determine whether consumption was significantly different than zero (H_o: $\mu = 0$) (Table 3-2). Homogeneity of variances for consumption among the different fluids was tested with Levene's Test. Variances were not homogeneous, so data were logarithmically transformed ($\log_{10}$ (x+1)). Transformed fluid consumption was compared via analysis of variance, and means were separated using Tukey's HSD (P = 0.05; SAS Institute 2000).

Results

Pharaoh ant workers foraged from the nesting cell to the microcapillary tubes in order to obtain food and water. No queens, larvae, or pupae were observed in the microcapillary tubes containing patient-care fluids or water. Workers were observed in microcapillary tubes within 5 min of placing fluids in test arenas. They generally entered and fed one at a time, due to the narrow tube diameter (1 mm ID), and exited by backing out. Occasionally, an extremely small worker was able to turn around within a microcapillary tube. In general, ants waited around the opening of the microcapillary tube, sometimes entering immediately behind another ant. When this occurred, all ants had to exit in reverse order to permit first ant to exit the tube. In relatively few instances, ants became stuck in the fluid and were unable to extricate themselves. Because stuck ants became embedded, the fluid spread out and extended past the stuck ant so that other ants were still able to feed upon the fluid. Numbers of ants remaining in fluids were not

recorded. Although some ants died during the starvation and the 24 h test period, mortality was not counted and was considered a natural event that did not significantly affect mean consumption per arena of any specific fluid.

A volume of ~21.2 µl of each patient-care fluid was provided. Because the tested fluids have differing specific gravities and because of minor variations in the volumes provided, initial weights were significantly different (Table 3-1). Ensure®, the most viscous fluid, had significantly more mass (~76 mg) placed in microcapillary tubes than other fluids. Saline had the least mass of fluid provided (~68 mg). Mean data for initial amounts of water provided in a separate study was measured (Table 3-1) but was not analyzed with other fluids.

Evaporation of the tested patient-care fluids from the microcapillary tubes was distinctly measurable despite the higher than room humidity in covered arenas containing cotton-stoppered water vials. Fluid loss in control arenas, due to evaporation only, ranged from 7-13 mg and did not differ significantly among the patient-care fluids (F = 2.10, df = 1, 80; P = 0.0879) (Table 3-1, Fig. 3-2). In contrast, there were significant differences in fluid loss of test arenas (Table 3-1), which was due to combined evaporation and consumption. Total fluid loss of dextrose (42.88 ± 2.36 mg) in test arenas was significantly higher than that of all other fluids; saline solution lost significantly less fluid (16.37 ± 3.60 mg) than all other fluids.

Temperature observations of 13 paired test units in May 2003, indicated that temperature inside covered test arenas (27.36 $\pm$ 0.31°C) was statistically significantly higher than inside covered control arenas (26.38 $\pm$ 0.34°C) and room temperature (26.40°C) by about 1°C, (F = 4.58; df = 1,25; P = 0.0428). The mean temperature of

control arenas was not significantly different than room temperature. Humidity was not significantly different between the insides of covered test (51.69 $\pm$ 0.88% RH) and control arenas (51.77 $\pm$ 1.34% RH) ($F = 0.00$; df = 1, 25; $P = 0.9621$), although they were both significantly higher than room humidity (38% RH).

Pharaoh ants consumed significant quantities of all patient-care fluids (Table 3-2). Consumption of 5% dextrose (37.95 $\pm$ 5.21 mg) was significantly higher than the consumption of all other fluids ($F = 14.86$; df = 4, 80; $P < 0.0001$; SAS Institute 2000). Consumption of human plasma (17.63 $\pm$ 3.26 mg), human blood cells (10.33 $\pm$ 1.67 mg), saline solution (9.94 $\pm$ 3.60 mg) and Ensure® dietary supplement (7.39 $\pm$ 1.53 mg) (i.e., all fluids except dextrose) were not consumed significantly differently. Consumption of baseline water controls (2.54 $\pm$ 0.54 mg) was significantly greater than zero (H_o: $\mu = 0$).

Discussion

Pharaoh ants must forage to obtain energy and protein to feed queen and larvae. Adult workers consume larval postpharyngeal regurgitations and anal secretions to help meet their own metabolic and nutritional needs (Hölldobler and Wilson 1990). Since pupae do not feed, development of Pharaoh ants must be nutritionally supported entirely during larval stages. Adult Pharaoh ants ingest to crop only particles smaller than 1.8 μm diameter (Petti 1998). Larger particles are filtered into the infrabuccal pocket and transported back to the immobile larvae in the nest (Petti 1998), where the larvae can digest these solid particles and later regurgitate fluids back to the workers and queens, primarily through the labial glands (Hölldobler and Wilson 1990).Since larvae provide digested food to workers and provide up to 93% of substances that queens depend upon (Börgesen and Jensen 1995), colony vigor depends on healthy growing of larvae. Larvae

are especially important in Pharaoh ant colonies, because the ability of queens to found new colonies is positively linked to larval presence (Börgesen and Jensen 1995). Protein is a necessary constituent of cuticle and organ development; if protein availability is limited, many species of ants will consume brood (Hölldobler and Wilson 1990). Peacock (1950) attributed brood mortality to various causes, including cannibalism of sexual brood. In general, the priority for protein feeding is queens, larvae and workers (Berndt 1977, Börgesen and Jensen 1995). Workers are last in the protein nutritional hierarchy, since queens and brood need large amounts for egg-laying and developing, respectively (Hölldobler and Wilson 1990).

Fluid Measurements

The amount of fluid provided can impact the rate of evaporation of any given fluid, since evaporation rates are dependent upon the volume of fluid present as well as upon the surface area exposed to air. Despite some fluctuation in temperature and humidity, the surface area from which evaporation could have occurred was limited to two 1 mm diameter openings, and as a result there was no significant difference between evaporation of the various liquids tested.

Finally, ants did not eat until depletion of provided fluids during the 24 h study. Thus, while Waldbauer's (1968) formula is the standard for measuring consumption in nutritional ecology studies, it would probably have been as acceptable to use a straight subtraction of evaporation as an adjustment for consumption in this experimental set up.

Water Consumption

Water is an essential component of ant survival and reproduction (Chapman 1998). Foraging is a multi-step process for Pharaoh ant colonies (Sudd 1957b, 1960).

After discovery of water or food, foragers lay faranal pheromone trails as they quickly return to the nest (Jeanson et al. 2003) and recruit additional workers. In this experiment, workers discovered the water vials within 15 min of placement into arena at the beginning of the 24 h starvation period and established a trail from the water vial to the nesting cell. They maintained these trails during the entire starvation period so that recruitment to water vials was established prior to placement of fluid-loaded microcapillary tubes. Because faranal is a short-lived pheromone with a half life of ~9 min on clean plastic surfaces (Jeanson et al. 2003), Pharaoh ants must continuously deposit faranal on trails to maintain recruitment to desirable resources. At the end of the starvation period, a microcapillary tube containing fluid was placed in arena; workers discovered the new fluid source almost immediately. In the water baseline study, which consisted of placement of water-loaded microcapillary tubes into the arena along with *ad libitum* water in 15 ml vial, the choice available to ants was consumption of water via different mechanisms, either via the microcapillary tube centered in the arena, or via the vial placed in the opposing corner of the arena, rather than between different fluids.

Water consumption in the baseline study was significant, but appeared to be much less than consumption of all other fluids, indicating that while Pharaoh ants consumed water in microcapillary tubes, they probably obtained water primarily from water vials. Observations in all test arenas of multiple ants feeding at water vials, of only 1 or 2 ants feeding at microcapillary tubes, and maintenance of recruitment trails to water vials support this possibility.

Dextrose Consumption

Simple sugars such as dextrose are immediately digested without need to break dextrose down into simpler components, as is needed for more complex sugars such as sucrose, fructose and other sugars. In fact, it is possible that dextrose is digested more rapidly than glucose that contains both L-glucose and D-glucose, because L-glucose must be converted to D-glucose first for digestion. Dextrose is actually composed entirely of D-glucose molecules, the most easily digested enantiomer of glucose.

Consumption of dextrose in each arena was significantly higher than consumption of Ensure®, the only other high-sugar fluid provided in this experiment. Foraging studies revealed that Pharaoh ants are attracted to sugar food sources (Sudd 1960, Haack and Vinson 1990, Vail and Williams 1994, Vail 1996). It is possible that foraging needs of Pharaoh ants change with seasonal requirements in a manner similar to that of *Solenopsis invicta* (L.) (Porter and Tschinkel 1987). Dextrose is probably more attractive to Pharaoh ants than Ensure® for several reasons: (1) sugar is the only nutrient present, (2) sugar is present as D-glucose, the simplest and most easily digested and metabolized form possible, and (3) concentration of sugar in 5% dextrose solution has a lower viscosity to allow for easier extraction by adult workers, and may help satisfy moisture requirements as well as energy requirements.

Significant consumption (H_o: $\mu = 0$) of dextrose agrees with research of other scientists who previously reported that ants feed readily and frequently on sugars. Many researchers used sugar or honey in either liquid solutions or as solids in bait and foraging studies for Pharaoh ants. Riley (1889) suggested coating pieces of crockery with sugar solution to attract Pharaoh ants, and Bellevoye (1889) reported Pharaoh ants consuming

sweets in his apartment and neighboring bakery. Therefore, it was not unexpected that consumption of 5% dextrose was significantly higher than consumption of other fluids.

Plasma and Blood Consumption

Pharaoh ants must forage for protein to meet metabolic needs of the queen for egg-laying and to enable larvae to complete their development. Human whole blood contains a significant amount of protein (~8%). Blood cells contain protein that is bound in bi-lipid cellular membranes and inter-cellular structures. In contrast, plasma contains most of whole blood's protein as free-floating particles in a solution that is 90% water.

Blood cells solidified in the microcapillary tubes during test, resulting in loss of capillary action and greatly increased surface area of exposed fluid. Although heparin slows coagulation of blood cells, blood cells solidified over time. Pipetting blood cells out of test tubes became increasingly difficult as samples aged. All samples were used within 7 d to reduce solidification. While heparin anticoagulant was present in whole blood samples, initial removal of plasma via pipette probably included removal of heparin, since plasma contains everything in whole blood except blood cells (AABB 2002). Therefore, blood cells as tested probably contained little anticoagulant during test period; thus, coagulation and solidification was more likely in blood cells than in plasma. Additionally, there was variability in coagulation levels between donated samples. Whole blood samples were not analyzed for hematocrit percentages (percentage of blood cells present in whole blood varies from person to person and even from day to day: i.e., hematocrit can increase when donor is dehydrated or decrease if donor has consumed large quantities of salt in diet) prior to use in this experiment.

The effect of heparin upon Pharaoh ant consumption was not studied in this experiment. There is evidence that heparin may negatively affect feeding ability of mosquitoes (Boyd and Kay 2000), but mosquitoes are capillary feeders specialized to feed directly from vertebrate host bloodstream (Service 2000, Mullen and Durden 2002), and heparin mostly affects the successful bloodmeal acquisition in mosquito (Boyd and Kay 2000). In contrast, Pharaoh ants are omnivorous feeders that feed upon exterior, open wounds rather than penetrating below vertebrate epidermis into sealed veins and arteries. Finally, ant colonies used in this experiment were small, thus, protein requirements may have been lower, resulting in less feeding of high-protein fluids.

In regards to both plasma and blood cells, this experiment represents the first time that blood, either as whole blood, or as its constituents, has been fed to Pharaoh ants, although Grace et al. (1986) fed human sputum to *Iridomyrmex (Linepithema) humilis* (Mayr) during a forensic murder investigation. While nobody has ever fed human plasma or human blood (or any animal plasma or blood) to Pharaoh ants, many researchers have determined that Pharaoh ants will feed on high-protein foods such as raw liver, liver powder, flies, and cockroaches (Bellevoye 1889, Sudd 1960, Edwards 1986, Haack and Vinson 1990). Therefore, significant consumption of human plasma, a high-protein fluid, comes as no surprise, and I suspect that presence of plasma in open wounds and at burn sites might be an important factor in Pharaoh ant attraction to patients in hospitals. In contrast, consumption of blood cells was not expected to be large, since blood cells are not as prevalent at open wound/burn sites as plasma. Burns tend to ooze plasma rather than to bleed, due to body's immune system response, which facilitates delivery of white

blood cells and enzymes (proteins) to damaged epithelium to fight infection and close open wounds by development of scar tissue.

Saline (=0.9% NaCl) Consumption

Electrolytes such as sodium (Na+) and chlorine (Cl-) ions (both present in saline solution) are nutrients that Pharaoh ants need in their diet (Chapman 1998). Vinson (1970) cited Butler's (1940) report that some dilute solutions of NaCl were preferred over water. However, significant consumption of saline solution in each test arena over a 24 h period after only 3 d of starvation was not expected. Saline was included because saline solution is often used in patient-care in hospital environments, both to replace lost fluids and to sterilize wounds, especially in burn units.

Saline consumption in this experiment agreed with Williams (1990) when he compared acceptability of saline to acceptability of several sugars (he used raw honey as test standard). It is interesting to note that the acceptance ratio he obtained for saline consumption was nearly identical to those for consumption of 10% dextrose and 10% glucose, both simple sugars. He contrasted saline's acceptance ratio (0.069 ± 0.015) against raw honey's acceptance ration (1.00 ± 0.000) to determine that while saline was accepted as a food source, it was much less preferable than most sugars.

Ensure® Consumption

Ensure® was the only test fluid that could be considered "complete," since it contains all three major nutrients: 26% proteins, 17% sugars, and 17% lipids. This fluid was expected to be highly attractive to Pharaoh ants and consumption of this complete food was anticipated to be higher than that of other fluids. Consumption of Ensure® may have been affected by its nutritional completeness. Vail reported that a bait containing

all three nutrients (carbohydrates, lipids and proteins) would attract any foraging Pharaoh ants. Although consumption of Ensure® was less than that of other fluids, that may reflect its completeness, since Pharaoh ants would not need to eat as much to meet their nutritional requirements. In contrast, they would need to consume large quantities of a low-density sole nutrient food to obtain the same energy and nutrition.

This experiment represents the first time that Ensure® (or any dietary supplement) has been provided to Pharaoh ants for consumption. These results provide some interesting information about consumption of complete foods vs. simpler foods.

It is possible that ant preference for Ensure® might have been limited for one or more reasons: I observed that Ensure® dried and partially solidified inside microcapillary tubes while other fluids remained in original liquid form. Solidification of Ensure® was most closely matched by solidification of blood cells, but the manner of drying was observed to be different. Specifically, blood cells lost microcapillary function and spread out in a thin layer along microcapillary tube bottoms after solidification and desiccation. In contrast, Ensure® dried in microcapillary tubes in such a manner as to partially block the tube, making ant penetration and extraction of fluid potentially more difficult.

Low consumption of plasma, blood cells and Ensure® might have been due to the presence of heparin anticoagulant or to high lipid content in high-protein fluids. Heparin was present in whole blood samples prior to centrifugation, so proportions of heparin in separated blood cells and plasma were unknown, and the effect of heparin on feeding was not studied. Protein comprises approximately 8% of plasma, 8% of blood cells (AABB 2002), and 17% of Ensure® (nutrition label). Previous research has shown that Pharaoh ants deplete protein stores as rapidly as carbohydrate stores (Haack and Vinson 1990,

Haack et al. 1995), but proteins used in those experiments consisted of a powdered egg yolk paste, and egg yolks have a high fat content of ~74% in contrast to their protein content of ~20% (USDA 2002). Ensure® has a much lower fat content (~17%) and a slightly higher protein content (~26%) than egg yolks. Pharaoh ants have fed upon lipid foods with no starvation (Williams 1990), so the effect of lipids upon consumption of Ensure® is unknown.

It is clear that in this study dextrose consumption exceeded that of all other fluids. Sumpter and Beekman (2003) reported Pharaoh ants preferentially recruit to foods with highest sucrose concentration. Knowing that Pharaoh ants are attracted to and will consume fluids used in patient-care, especially dextrose, hospital staff should not spread these fluids in hospital; if these fluids are present (especially near patient), they should be cleaned up quickly to decrease potential for Pharaoh ant attraction to patients within hospital and other health-care environments.

Table 3-1. Mean (liquid no-choice) initial weights of fluid quantity provided (mg) and mean fluid loss (mg) during test period (24 h) for each of five different patient-care fluids used in hospital environments. Test arenas with ants (n=250); control arenas lacked ants.

	Mean (mg) ± SEM			
	Control arenas		Test arenas	
Patient-care Fluid	Initial wt[a]	Fluid Loss (Evap.)[a,b]	Initial wt[a]	Fluid Loss[a,b,c]
Blood	72.77 0.29b	12.71 ± 1.90a	72.88 ± 0.28b	21.97 ± 2.36abc
5% Dextrose	69.75 ± 0.26cd	9.25 ± 2.14a	69.71 ± 0.26c	42.88 ± 5.16a
Ensure®	77.36 ± 0.41a	8.59 ± 0.52a	76.59 ± 0.37a	15.34 ± 1.56bc
0.9% NaCl	68.68 ± 0.33d	7.54 ± 1.00a	68.39 ± 0.47d	16.37 ± 3.60c
Plasma	70.38 ± 0.24c	13.53 ± 2.34a	70.64 ± 0.24c	28.42 ± 3.60ab
Water[d]	69.48 ± 0.12	3.21 ± 0.19	69.54 ± 0.16	5.64 ± 0.59

[a] Means within a column followed by the same letter are not significantly different by Tukey's HSD test ($P = 0.05$).
[b] Fluid Loss in control arenas is to evaporation only.
[c] Fluid loss in test arenas due to evaporation and consumption combined.
[d] Water analysis done separately as baseline study; water data not part of test fluid analysis.

Table 3-2. Mean (liquid no-choice) consumption (24 h) for each of five different patient-care fluids used in hospital environments by starved (3d) Pharaoh ant colonies (n=250).

Patient-care Fluid	Test arenas Mean consumption ± SEM (mg)	df	*t*	*P*
Blood	10.33 ± 1.67b	16	6.17	< 0.0001
5% Dextrose	37.95 ± 5.21a	16	7.28	< 0.0001
Ensure®	7.39 ± 1.53b	16	4.83	< 0.0001
0.9% NaCl	9.94 ± 3.60b	16	2.76	0.0095
Plasma	17.63 ± 3.26b	16	5.41	< 0.0001
Water[a]	0.48 ± 0.07	16	7.25	< 0.0001

Mean consumption within a row (H_0: $\mu = 0$) analyzed via Student's *t*-test (SAS Institute 2000). Mean consumption of fluids (mg) and rank (number) listed in columns analyzed via Tukey's HSD test ($P = 0.05$). Means within a column followed by same letters are not significantly different ($F = 14.86$; df = 4, 80; $P < 0.0001$); data were logarithmic-transformed before analysis.

[a] Water analysis done separately as baseline study; water data not part of test fluid analysis.

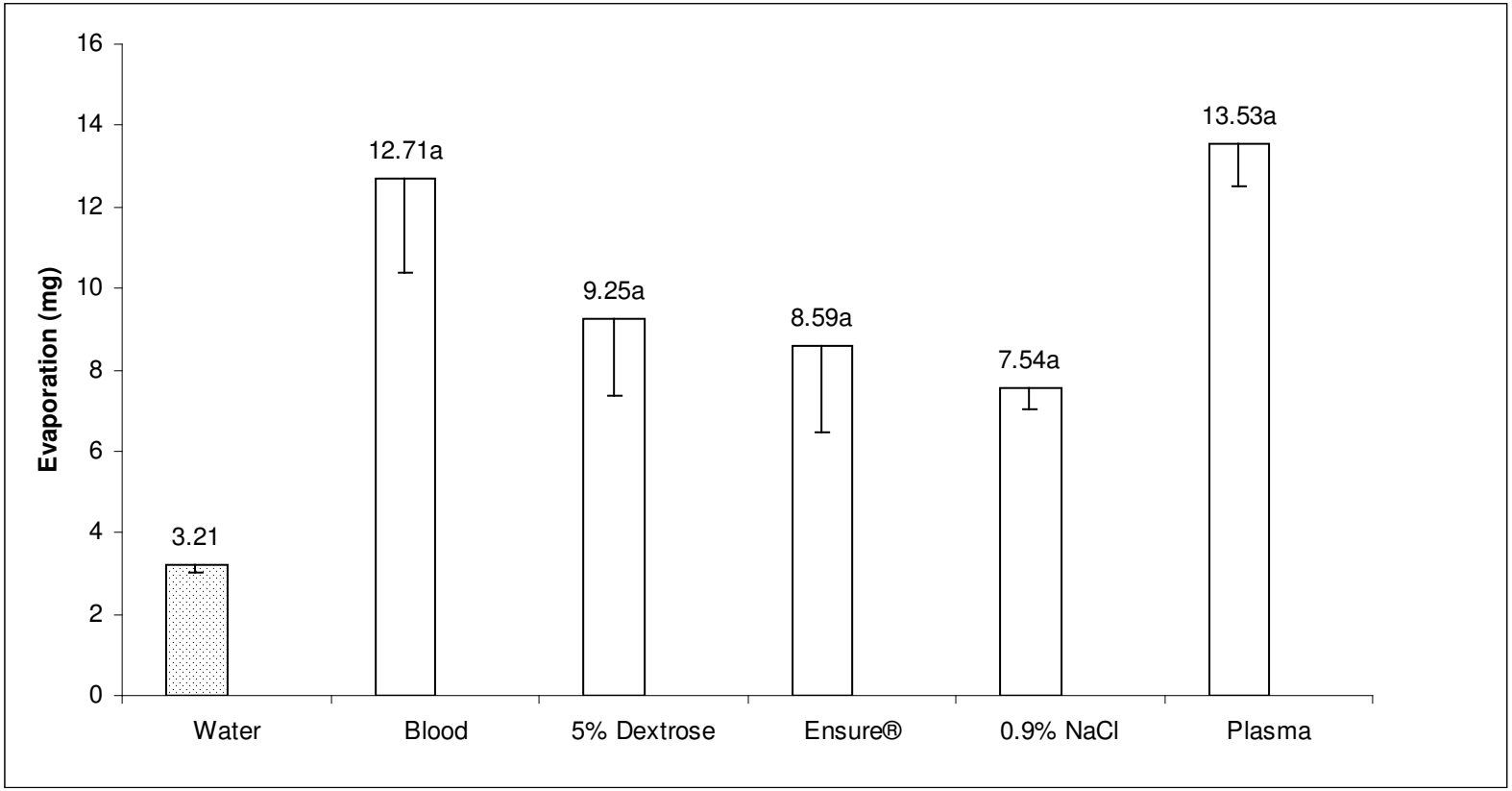

Figure 3-2. Mean (liquid no-choice) evaporation (24 h) in control arenas for each tested patient-care fluid used in hospital environments. Evaporation means followed by same letter are not significantly different (Tukey's *t*-test; F = 2.10; df = 4, 80; $P < 0.0001$). Water analysis done separately as baseline study; water data not part of test fluid analysis

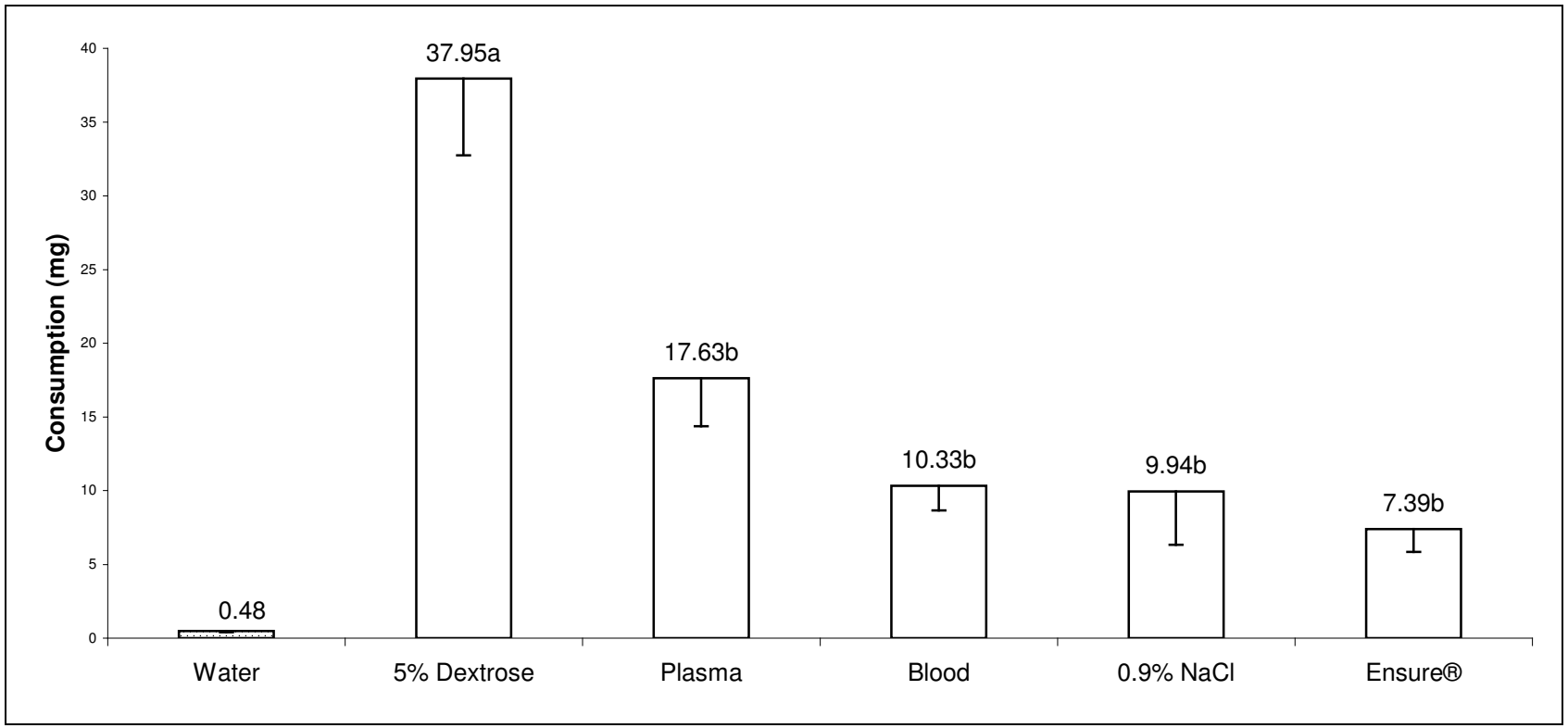

Figure 3-3. Mean (liquid no-choice) consumption (24 h) in test arenas for each tested patient-care fluid used in hospital by starved (3d) Pharaoh ant colonies (n=250). Means followed by same letter are not significantly different (Tukey's *t*-test; F =13.97; df = 4, 80; $P < 0.0001$); data were logarithmic-transformed before analysis. Water analysis done separately as baseline study; water data not part of test fluid analysis..

CHAPTER 4
CHOICE LIQUID TEST

Introduction

Many social insects are omnivores, consuming a wide variety of foods from multiple nutritional classifications. This is especially true of hymenopteran colonies. Within the hymenoptera, omnivorous diets are frequently seen in many species of ants (Hölldobler and Wilson 1990). This omnivorous diet differentiates them from other social insects such as termites which are specialized to feed upon foods that are limited in their availability, either nutritionally (dead wood in the case of termites) or seasonally (pollen and nectar in the case of bees) (Wheeler 1910).

Like most other tramp ant species, Pharaoh ants feed on a wide variety of energy-rich foods (Wheeler 1910) that include insects, nectar, fruit, dead animals, sweets, pastries, meat, jelly, (Peacock et al. 1950, Sudd 1960). Disturbingly, Pharaoh ants also feed on humans (Beatson 1972, Edwards and Short 1990, Eichler 1990)

Nutritional needs of social insects are complex and varied due to the structure of their colonies. Ant colonies contain different castes and development stages: workers and reproductives, eggs, larvae, and pupae; all stages except eggs and pupae have nutritional needs and consume food. Nutritional needs differ among castes and stages: for example, queens need more protein for egg-laying, larvae need protein to complete development, and workers require more carbohydrates to supply energy for foraging and tending of brood and queens (Hölldobler and Wilson 1990). The nutritional needs of the colony reflect the combination of all individual members' nutritional needs within the

colony. Cassill and Tschinkel (1999) reported that the overlapping nutritional needs of different caste members determines foraging and nursing behavior by worker ants within the colony.

An interesting aspect of Pharaoh ants is their ability to change their consumption to adjust for the nutritional need of the colony. Edwards and Abraham (1990) demonstrated that Pharaoh ants alternate between high-protein and high-carbohydrate foods. They concluded that foods containing both of these nutrients would prove more attractive as Pharaoh ant bait matrices, since inclusion of both simultaneously would provide the ants with both highly-recruited nutrients. They also noted that Pharaoh ant colonies which have satiated upon one nutrient, (e.g. carbohydrates) might recruit more to the other major nutrient, such as protein. Because Pharaoh ants vary their diet, their foraging and feeding behavior is an especially important consideration when alternative foods are available for consumption.

In hospitals, a wide variety of food is available throughout the hospital, in kitchens and cafeterias, in food-carts wheeled through the hospital, and in snack vending machines. In addition, Pharaoh ants have access to unique nutritional fluids, such as human body fluids and fluids used in patient-care, available to them in health-care environments. Pharaoh ants have been observed feeding around wounds and body openings, which might have been leaking body fluid (Eichler 1990). Pharaoh ant consumption of fluids associated with hospital patients was confirmed in a no-choice bioassay (Chapter 3). Because patient-care fluids are used throughout the hospital, further examination was deemed necessary to determine how the simultaneous availability of these patient-care fluids might affect Pharaoh ant consumption.

Specifically, the objective was to determine the preference of small colonies of Pharaoh ants for five liquid patient-care fluids using a choice test.

Materials and Methods

Ants

Pharaoh ant test colonies were extracted from large parent colonies housed and reared at the Urban Entomology Laboratory, University of Florida, Gainesville, FL. Rearing temperatures ranged from 21.9°C – 29.4°C with relative humidity between 20-53%. (Appendix A) Colonies consisted of 250 workers, 3 dealate queens and approximately 50 mg brood. One hundred twenty five workers were selected from cells to ensure the presence of nurses and 125 workers were selected from outside cells to ensure the presence of foragers (Haack 1987, Haack and Vinson 1990, Haack et al. 1995). Brood included eggs, all larval stages and pupae. See Appendix B for full details on selection of ants and preparation of experimental test colonies.

Foraging Arena

Foraging arenas contained one nesting cell, one water vial and five microcapillary tubes with fluids (Figure 4-1). These arenas were identical to those used in the previous no-choice liquid consumption test (Chapter 3) except for the addition of four microcapillary tubes to permit fluid availability. A detailed description of the foraging arena was provided in Chapter 3.

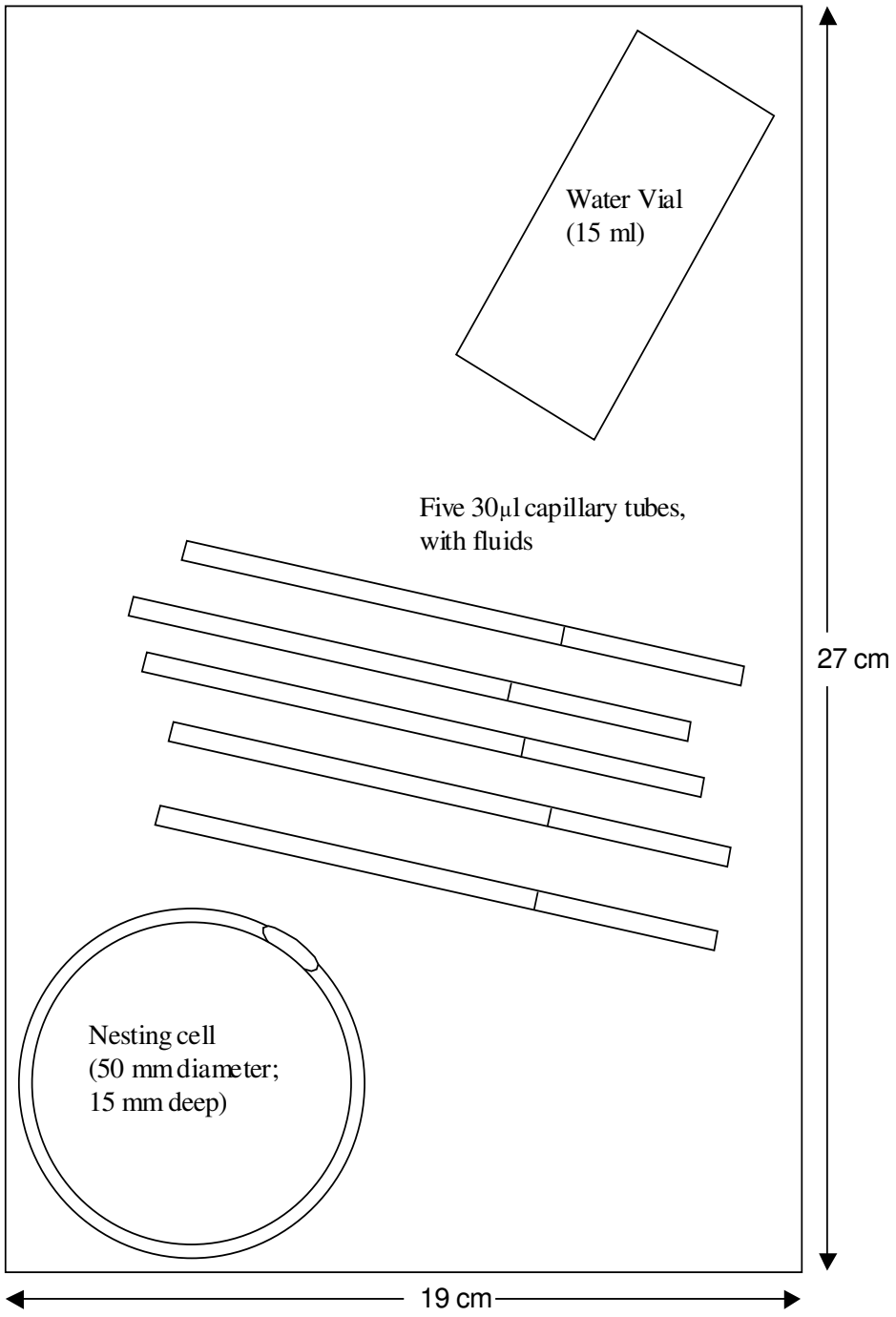

Figure 4-1. Arenas with water vial and nesting cells placed in diagonally opposite corners. Capillary tubes placed in center. Test arenas (with ants) and control arenas (no ants) were placed adjacent to each other and comprised a test pair. Test arenas contained 125 inside ants, 125 outside ants, 3 queens and ~50 mg brood (including all stages of brood).

Water was provided *ad libitum* in a 15 ml vial (Model No. 55-9, Thornton Plastics, Salt Lake City, UT) with three large cotton balls inserted into top to prevent leakage and to provide easy access for ants. A water vial was placed in the opposite diagonal corner of arena across from the nesting cell. Each replicate consisted of one test arena placed adjacent to one control arena. Test arenas contained ants, while control arenas lacked ants. Control arena data was used to quantify and adjust for evaporation of fluids. Each arena was covered with a clear plastic lid to maintain humidity and temperature.

Experimental Fluids

The same five fluids used in the no-choice test in Chapter 3 were used in this choice test. Fluids consisted of human blood cells, human plasma, saline (0.9% sodium chloride); 5% dextrose (D-glucose), and Ensure® (Vanilla Plus, Ross Products Division, Abbott Laboratories, Columbus, OH). Fluids were chosen to replicate hospital conditions and potential fluid sources as closely as possible. The detailed descriptions of each fluid and handling procedures used in this study are identical to that provided in Chapter 3.

Experimental Design

The experiment was designed as a randomized complete block, blocking on colony. Each block consisted of one Pharaoh ant colony (125 inside workers, 125 outside workers, 3 dealate queens and ~50 mg brood) obtained from a single parent colony. Five fluids were simultaneously offered to each colony to determine the consumption among the test fluids under a choice exposure. As in the previous no-choice experiment (Chapter 3), an experimental unit consisted of a paired test and control arena located adjacent to one another. The choice test was replicated 21 times. Ants were selected from 6 different parent colonies. Fluids were tested over a two week period from May

21, 2003 to June 3, 2003; room temperature and relative humidity were maintained between 24.9-28.6°C and 35-57% RH.

Pharaoh ant colonies were starved for 48 h; water was available *ad libitum* in all arenas, including water controls, via a 15 ml cotton-stoppered vial during starvation period (Haack and Vinson 1990, Haack et al. 1995). After 48 h of starvation, five microcapillary tubes, each containing one test fluid, were added to each arena, and the water vial was not removed. Thus, water was always available in all arenas. Fluids were offered simultaneously to each colony to determine consumption preference among provided fluids.

Mortality after starvation period and prior to 24 h feeding period in test arenas was observed, but not recorded. Dead ants were neither counted nor removed. Mortality rates during starvation periods were not taken into consideration when recording consumption from test arenas.

Fluid Measurements

Fluid placement into arenas was identical to the previous no-choice test (Chapter 3) except that five different fluids were simultaneously provided, each in its own microcapillary tube. In brief, each microcapillary tube (Model No. 21-380-9C, Fisher Scientific, Pittsburgh, PA) was weighed before and after the liquid was loaded. The amount of liquid provided to all control and test arenas was calculated for each arena by subtracting the mass of the empty microcapillary tube from the mass of the tube containing initial mass of provided fluid (Wei and Johnson 1996).

Ants were allowed to feed for 24 h. Microcapillary tubes were then reweighed in both control and test arenas. Due to frequent presence of ants still in microcapillary tubes during final gravimetric measurement on a digital scale, weights of post-consumption test

fluid after 24 hour test period included the mass of ants remaining in microcapillary tubes. Final ant biomass measurements were included in final mass weights recorded and were subtracted later using an average mass value of 0.0002 g per Pharaoh ant worker. Worker ant mass was determined by weighing several individual worker ants to determine average mass per ant.

Fluid loss in control arenas was calculated by subtracting amount of fluid initially provided from amount of fluid remaining, resulting in the amount of evaporation that occurred in the 24 h test period. Fluid loss in test arenas was comprised of consumption and evaporation; therefore, to determine consumed quantities, the amount of fluid lost to evaporation was subtracted. Many researchers have designed formulas to adjust consumption for fluid loss, and Waldbauer's (1968) formula was selected (Appendix C). Because multiple ants remained in test arena fluids, Waldbauer's (1968) formula was modified to account for mass of ants remaining in fluid. Thus, corrected consumption was determined as follows:

$$C = (1 - \tfrac{1}{2}[Y/G]) * (W - [(L - A) + (Y/H)(L - A)])$$

where

C= corrected consumption,

W = mass of fluid provided (g) in test arena,

L = mass of uneaten fluid and ants (g) in test arena,

A = n*(0.0002 g/ant) mass of ants stuck in tube, n = number of ants stuck in tube,

thus, (L-A) = mass of uneaten fluid,

G = mass of fluid provided (g) in control arena,

H = mass of fluid remaining (g) in control arena.

Y = G – H = weight loss (g) of control fluid,

Y/G = weight loss of control fluid / initial mass of control fluid (= a, in Waldbauer 1968),

Y/H = weight loss of control fluid / final mass of control fluid (= b, in Waldbauer 1968).

Statistical Analysis

Dead or alive ants remaining in microcapillary tubes at the conclusion of the 24 h test period were compared among test fluids. Mean number (n) of ants remaining in test fluids was compared via analysis of variance and Tukey's HSD test ($P = 0.05$; SAS Institute 2000)

Fluid loss from evaporation in the control arenas and fluid loss from evaporation and consumption in the test arenas among types of hospital fluids were compared by analysis of variance and Tukey's HSD ($P = 0.05$; SAS Institute 2000). Variances were tested using Levene's Homogeneity of Variances test ($P = 0.05$; SAS Institute 2000).

Negative consumption values (15 of 100 values) were truncated to zero. Consumption of patient-care fluids and water was analyzed via a one-tailed Student's *t*-test to determine if there was significant consumption (H_o: $\mu = 0$).

Consumption data were compared via analysis of variance, and means were separated using Tukey's HSD test ($P = 0.05$; SAS Institute 2000). Homogeneity of variances for consumption among the different fluids was tested with Levene's Test ($P = 0.05$; SAS Institute 2000).

Results

Number of Ants

Ants were observed entering microcapillary tubes within 5 min of placing fluids in test arenas. Observations of ant behavior in this experiment were similar to those seen in Chapter 3.

Mean number of ants remaining in Ensure® (5.77 $\pm$ 0.96) at the conclusion of the test period was significantly higher (F =12.40; df = 4, 95; $P < 0.0001$) than in the other fluids (Table 4-1). Numbers of ants remaining in all other fluids were low and did not significantly differ, ranging from 0.71 to 1.94 (Fig. 4-2).

Fluid Measurements

Initial quantities of fluids provided to test and control arenas differed significantly (F = 256.9, df=4, 95; $P < 0.0001$) among the tested patient-care fluids. The mass of Ensure® (~77 mg) placed into control arenas was higher than that of all other fluids. The mass of blood (~76 mg) was significantly higher than that of plasma, dextrose, and saline. The mass of provided saline (~69 mg) was less than that of all other fluids in control arenas, but not in test arenas (Table 4-2).

In test arenas, initial amounts of fluids provided were also significantly different (F = 133.39; df=4, 95; $P <0.0001$). As in control arenas, significantly more Ensure® by mass was placed into arenas than all other fluids, the mass of blood provided was significantly higher than that of plasma, dextrose, and saline, and the mass of provided saline was significantly less than that of all other fluids (Table 4-2).

Evaporation in control arenas was significantly different between fluids (F = 4.85; df = 4, 95; P= 0.0013). Plasma had the highest fluid loss due to evaporation, while saline and dextrose had the least amount of fluid loss due to evaporation (Table 4-2).

Fluid loss of tested patient-care fluids in test arenas was significantly different due to combined evaporation and consumption (F = 29.01; df=4, 95; $P < 0.0001$). When ants were present, significantly more dextrose (~77 mg) was lost than any other fluid. Ensure® lost more fluid than plasma, saline and blood. Saline and blood had the smallest amount of fluid loss (Table 4–2). Fluid loss in control arenas was due solely to

evaporation, and ranged from 2.59 ± 0.19 mg for dextrose to 4.87 ± 0.68 mg for plasma. In contrast, fluid loss in test arenas ranged from 5.20 ± 0.58 mg for blood to 50.80 ± 3.46 mg for dextrose (Fig. 4-3, Table 4-2). Temperatures in test arenas (27.36 ± 0.31°C) were statistically significantly higher (F=4.58; df = 1, 24; P = 0.0428) than in control arenas (26.38 ± 0.34°C), probably due to the presence of ants.

Consumption

There were significant differences (F = 29.97; df = 4, 95; P < 0.0001) in consumption of the fluids by Pharaoh ants. Consumption of 5% dextrose (49.14 ± 3.51 mg) was significantly higher than consumption of all other fluids. Consumption of Ensure® (16.05 ± 6.23 mg) was significantly greater than consumption of plasma (9.31 ± 3.17 mg), saline (3.09 ± 1.14 mg), and blood cells (2.46 ± 0.48 mg) (Table 4-2, Fig. 4-3). Baseline mean consumption of water (0.48 ± 0.07 mg) was lower than the consumption of all test fluids. None of the fluids in any of the microcapillary tubes in either the test or control arenas were completely depleted.

Discussion

Number of Ants

Numbers of ants remaining in microcapillary tubes at conclusion of test period did not correlate to heaviest consumption or to observed foraging behavior. Ants remaining in microcapillary tubes appeared to be feeding on remaining fluid. Foraging activity observed during the first hour of the test period was of largest recruitment to the microcapillary tubes containing 5% dextrose.

In contrast, the Ensure had the highest number of ants remaining in the tubes after 24 h. The mean number of ants remaining in microcapillary tubes after 24 h within each arena comprised 6% of colony workers (n=250) (Fig. 4-1).

Fluid Measurements

The significant differences in mass between different fluids probably reflects the different densities of the fluids used in this experiment, since all microcapillary tubes were filled to the same volume of ~21.2 µl. Higher initial masses for both Ensure® and blood were associated with the higher viscosity observed in these two fluids. Similarly, saline solution contained only 0.9% electrolytes, making it the least viscous fluid used.

Differing amounts of provided fluid did not affect consumption since no fluid was depleted. Differing amounts of provided fluid (Table 4-2) might have resulted in different rates of fluid loss for different fluids. Although Ensure® had significantly more fluid mass provided in both control and test arenas than other fluids, evaporation of Ensure® in control arenas did not differ from that of other test fluids.

Fluid loss in test arenas (Table 4-2) was comprised of both consumption and evaporation; therefore, the amount of fluid lost to evaporation had to be subtracted in order to determine consumption values.

Although temperatures within test arenas were statistically significantly higher than in control arenas, both means were within 1°C of temperatures reported as ideal for Pharaoh ants (Peacock and Baxter 1949, Peacock et al. 1955c). Hence, relative to temperature, foraging was probably optimal in this experiment. Humidity did not differ between control and test arenas; however, the humidity levels inside arenas of ~52% were well below the 80% recommended by (Peacock and Baxter 1949, Peacock et al. 1955c) and also lower than the 65% humidity recommended by (Haack 1987) as optimal for the Pharaoh ant. Low humidity probably increased the moisture needs of the test colonies, and probably increased the rate of evaporation of all fluids. If low humidity

resulted in an increased moisture requirement, then the consumption of fluids may have increased.

Consumption

(Mailleux et al. 2000) determined that ant foraging behavior is a response to the nutritional demands of the ants and to the characteristics of the food resources, such as the density and spatial distribution, distance to food, and the quality of food. Consumption of high-carbohydrate and high-protein fluids was expected, since a nutritional need was assumed to be induced by a 3 d starvation period. Food characteristics affecting ant foraging behavior varied only in the nutritional composition and quality of the different fluids offered, since all microcapillary tubes were placed randomly in replications (Mailleux et al. 2000).

In this experiment, all fluids were consumed in significant proportions, indicating that the ants consumed a wide variety of foods when provided with a choice. This agrees with observations that Pharaoh ants change food selections based on satiety and preference, and that they will even alternate between high-carbohydrate and high-protein food sources (Granovsky and Howell 1983, Edwards and Abraham 1990).

Consumption also can be influenced by colony needs, and can change depending on a variety of factors such as colony size, proportion of larvae to workers, and numbers of egg-laying queens. For example, egg-laying by queens and larger numbers of larvae both increase the colony's need for proteins. In the red imported fire ant, *Solenopsis invicta* (Buren), even worker size or crop contents can have an impact on food flow within a colony's members (Cassill and Tschinkel 1999).

Dextrose

Pharaoh ants have been reported to foraging most strongly to fluids with highest sugar concentrations (Sumpter and Beekman 2003), such as sucrose solutions. While the Pharaoh ants consumed all fluids, 5% dextrose was consumed significantly more than all other liquids (Fig. 4-3). Mean consumption of 5% dextrose (49.14 $\pm$ 3.51 mg) was consumed 3 times more than Ensure®, than the next most consumed fluid (Table 4-3).

These results were similar to those obtained in the no-choice test (Chapter 3). Haack et al. (1995) reported a similarly high Pharaoh ant recruitment to simple carbohydrates: he observed that ~100% of workers foraged at carbohydrate source within ~3 h of introduction of food into arena, while only 2% foraged to proteins within 24 h, and ~80% foraged to lipids after 24 h. In hospitals, 5% dextrose is often squirted onto floors when nurses or doctors clear intravenous tubing prior to inserting tubing into patients. The amount of time that might elapse between spillage and cleanup is unknown; if dextrose is present in the rooms, this experiment clearly indicates that Pharaoh ants will recruit to this fluid.

Ensure®

When given a choice of fluids after 3 d starvation, Pharaoh ants preferred high-sugar fluids most; the high concentration of sugars in Ensure® probably helped make it the second most-preferred fluid in this experiment, despite its relatively high evaporation rate (Fig. 4-4) and tendency to dry out and partially solidify, thus making it more difficult to extract than the less viscous dextrose.

The relatively high consumption of Ensure® in this choice test contrasted with the lesser consumption of Ensure® in the no-choice test (Chapter 3). Pharaoh ants preferred fluids with high concentrations of sugar. Although Pharaoh ant consumption of Ensure®

was second only to consumption of dextrose, it was significantly less than that of dextrose. Ensure® has fewer simple sugars, and more complex sugars than dextrose, making it a more complex fluid. Insects rapidly convert ingested glucose in trehalose for storage in hemolymph, and must take an additional, high-energy step of converting complex sugars into glucose first (Nation 2003). Ensure® dietary supplement is further complicated since it contains high concentrations of proteins and lipids in addition to sugars.

Finally, Ensure® had more ants stuck in it than all other fluids (Table 4-1, Fig. 4-2). Some ants remaining in fluid were dead during final weighing; Sudd (1957a) observed Pharaoh ants respond in a negative manner to dead Pharaoh ants. He reported that when a Pharaoh ant encountered dead of its own species, it became alarmed and ran away when within 1 cm of dead and; however, those ants were killed by crushing. In this experiment, most ants remaining in the fluid were still alive; ants which were dead had not been crushed. Any chemicals emitted via crushing that may have served as an alarm pheromone were probably not present in this experiment. Therefore, the presence of dead ants in the tubes was not expected to influence feeding behavior. Visual observation of ants in microcapillary tubes did not note any forage reluctance to enter tubes containing ants. Ultimately, the nutritional complexity and dead ant accumulation in Ensure® did not prevent foragers from removing significant quantities of fluid.

Plasma

Plasma evaporated at a significantly higher rate than all other fluids, and was the only fluid with a higher evaporation rate than Ensure®. While plasma evaporated at the highest rate, consumption of plasma (9.312 ± 3.17) was not significantly different than that of saline (3.093 ± 1.14). The high evaporation rate of plasma probably increased the

concentration of ions within the solution more rapidly than occurred in saline. The presence of heparin anticoagulant may have influenced ant consumption of plasma, and may have influenced the rate of evaporation as well.

Both human plasma and saline solution are very aqueous fluids which are high in sodium and chlorine electrolytes. In fact, the electrolyte content of 0.9% NaCl solution and human plasma are identical; saline is often used as a fluid replacement to increase blood volume (AABB 2002). So it was not surprising that Pharaoh ant preference for these two fluids was not statistically different.

NaCl

Saline solution is a very aqueous solution, composed mostly of water, with 0.9% sodium chloride ions. Evaporation of saline was the second lowest of tested fluids; evaporation of saline was higher than that of dextrose. Both fluids were highly aqueous solutions with low-density so that ant ingestion was easy. Consumption of saline was significant, but was less than that of high-sugar fluids.

Many electrolyte solutions are repellent to red imported fire ants (Vinson 1970). However, Vinson determined that the addition of salt increased the acceptance of sugars by red imported fire ants. Thus, the available choice of NaCl solution in test arenas may have resulted in increased consumption of high-sugar fluids, dextrose and Ensure®. In this experiment, Pharaoh ants consumed 0.9% NaCl solution (3.093 ± 1.14) despite the *ad libitum* presence of water. This clearly indicated that the Pharaoh ants were interested in saline solution for nutritional reasons, especially when baseline water consumption test results (0.48 ± 0.07 mg) were taken into account.

Blood

Blood cells were the least consumed of all tested fluids. Blood was the only fluid which had a higher rate of evaporation (3.32 $\pm$ 0.25) than of consumption (2.046 $\pm$ 0.48), although blood loss in the test arena (5.20 $\pm$ 0.58) was greater than evaporation (Table 4-2). Very little foraging activity was observed on microcapillary tubes containing blood cells. The ants did not recruit to it very strongly as a food source, although they did consume it in significant amounts. Solidification of the blood cells was observed. The small inner diameter of the microcapillary tube and a hardened, solidified surface probably made extraction very difficult for foragers.

Blood was one of the two most viscous fluids in this experiment, and consumption of very dense and highly-nutritious blood cells may have resulted in satiation at lower levels of consumption. Alternatively, blood cells are high in protein, but are not high in sugars, so the lower consumption of blood cells, as compared to that of high-sugar fluids dextrose and Ensure®, might have indicated that the Pharaoh ants' nutritional need for carbohydrates was higher than for protein during the test period.

It is clear that in this study, dextrose was preferred over all other fluids. It is possible that high sugar (sole nutrient) concentration is favorable for Pharaoh ant fluid consumption. Sumpter and Beekman (2003) reported Pharaoh ants preferentially recruit to foods with highest sucrose concentration. When ants are placed in test arena, they consumed all patient-care fluids. Knowing that Pharaoh ants are attracted to and will consume fluids used in patient-care, especially dextrose, hospital staff should not spread these fluids in hospital; if these fluids are present (especially near patient), they should be cleaned up quickly to decrease potential for Pharaoh ant attraction to patients within hospital and other health-care environments.

Table 4-1. Mean number of ants remaining (either feeding, stuck or dead) in fluids at end of 24 h choice test feeding period.

Liquid Food	Test arenas Mean number ants ± SE
Blood	0.71 ± 0.49b
5% Dextrose	1.06 ± 0.34b
Ensure®	5.77 ± 0.96a
0.9% NaCl	1.94 ± 0.56b
Plasma	1.12 ± 0.42b
Water[a]	2.77 ± 1.10

Means followed by same letter are not significantly different by Tukey's HSD-test (P = 0.05; SAS Institute 2000).

[a] Water analysis done separately as baseline study; water data not part of test fluid analysis.

Table 4-2. Mean (choice test) initial weights of fluid quantity provided (mg) and mean fluid loss (mg) for each of five different patient-care fluids used in hospital environments (24 h) for starved (3 d) pharaoh ant colonies.

Patient-care Fluid	Mean (mg) ± SEM			
	Control arenas		Test arenas	
	Initial wt	Fluid Loss[a]	Initial wt[a]	Fluid Loss[b]
Blood	75.81 ± 0.28b	3.32 ± 0.25ab	76.03 ± 0.42b	5.20 ± 0.58c
5% Dextrose	70.47 ± 0.17cd	2.59 ± 0.19b	70.51 ± 0.25cd	50.80 ± 3.46a
Ensure®	76.92 ± 0.18a	4.00 ± 0.24ab	77.25 ± 0.35a	19.62 ± 6.08b
0.9% NaCl	69.76 ± 0.13d	2.98 ± 0.48b	69.99 ± 0.18d	5.82 ± 1.16c
Plasma	71.15 ± 0.24c	4.87 ± 0.68a	71.48 ± 0.17c	13.07 ± 3.19bc
Water[c]	69.48 ± 0.12	3.21 ± 0.19	69.54 ± 0.16	5.64 ± 0.59

Means within a column followed by the same letter are not significantly different by Tukey's HSD-test ($P = 0.05$).
[a] Fluid Loss in control arenas is to evaporation only.
[b] Fluid loss in test arenas due to evaporation and consumption combined.
[c] Water analysis done separately as baseline study; water data not part of test fluid analysis.

Table 4-3. Mean (choice test) consumption (mg) for each of five different patient-care fluids used in hospital environments (24 h) for starved (3 d) pharaoh ant colonies.

Liquid Food	Test arenas Mean consumption ± SEM (mg)	df	*t*	*P*
Blood	2.046 ± 0.48c	38	4.23	0.0001
5% Dextrose	49.140 ± 3.51a	38	14.02	<0.0001
Ensure®	16.054 ± 6.23b	38	2.58	0.0056
0.9% NaCl	3.093 ± 1.14bc	38	2.71	0.0101
Plasma	9.312 ± 3.17bc	38	2.94	0.0056
Water[a]	0.48 ± 0.07	16	7.25	<0.0001

Mean consumption within a row (H_0: $\mu = 0$) analyzed via Student's *t*-test ($P = 0.05$; SAS Institute 2000).
Mean consumption of fluids (mg) listed in columns analyzed via Tukey's means separation test ($P = 0.05$).
Means within a column followed by same letters are not significantly different by Tukey's HSD-test ($P = 0.05$).
[a] Water analysis done separately as baseline study; water data not part of test fluid analysis.

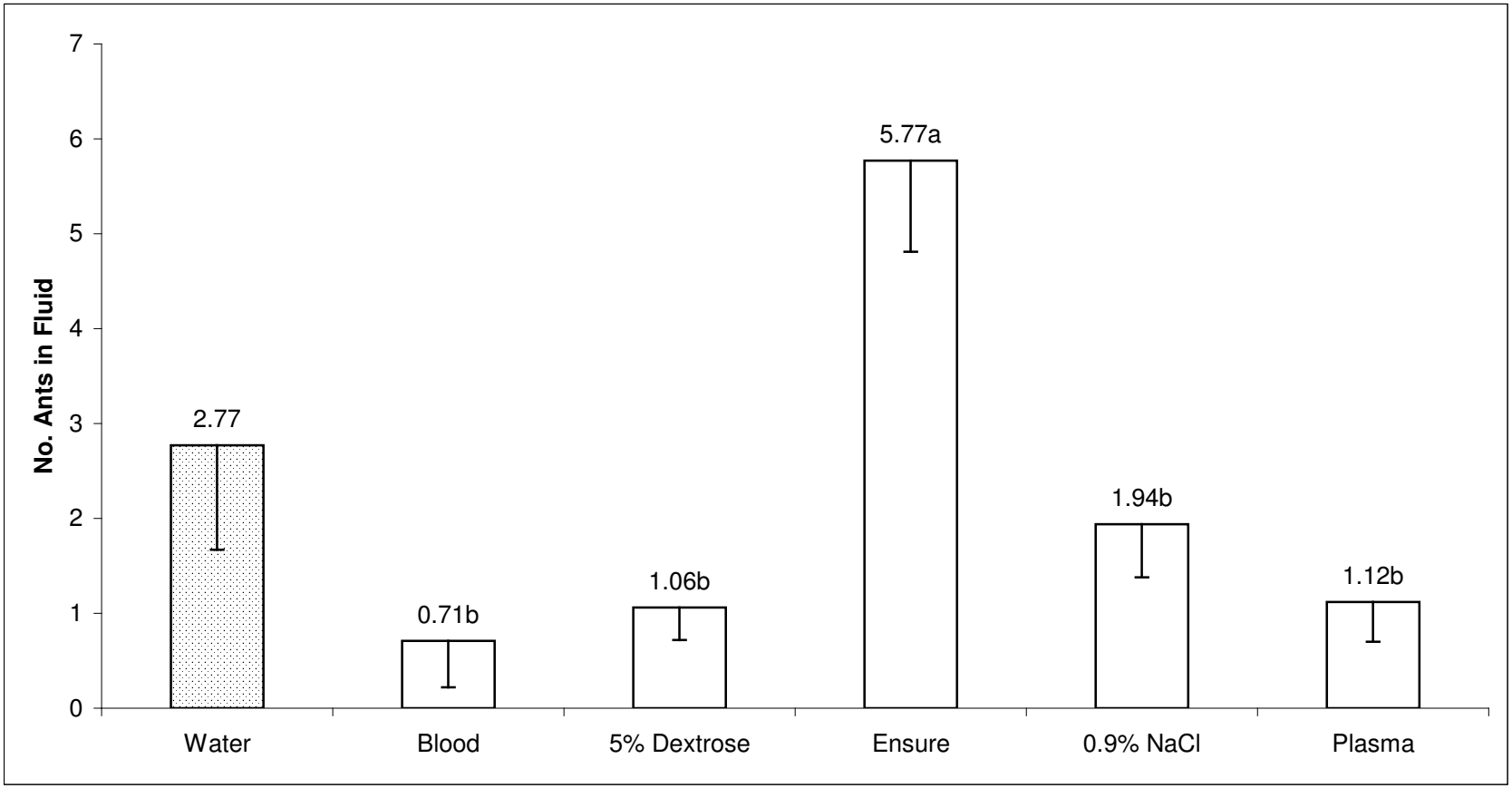

Figure 4-2. Mean number of ants (either dead or feeding) remaining in fluid at end of 24 h choice test feeding period. Means followed by same letter are not significantly different by Tukey's HSD-test ($P = 0.05$; SAS Institute 2000). Water analysis done separately as baseline study; water data not part of test fluid analysis.

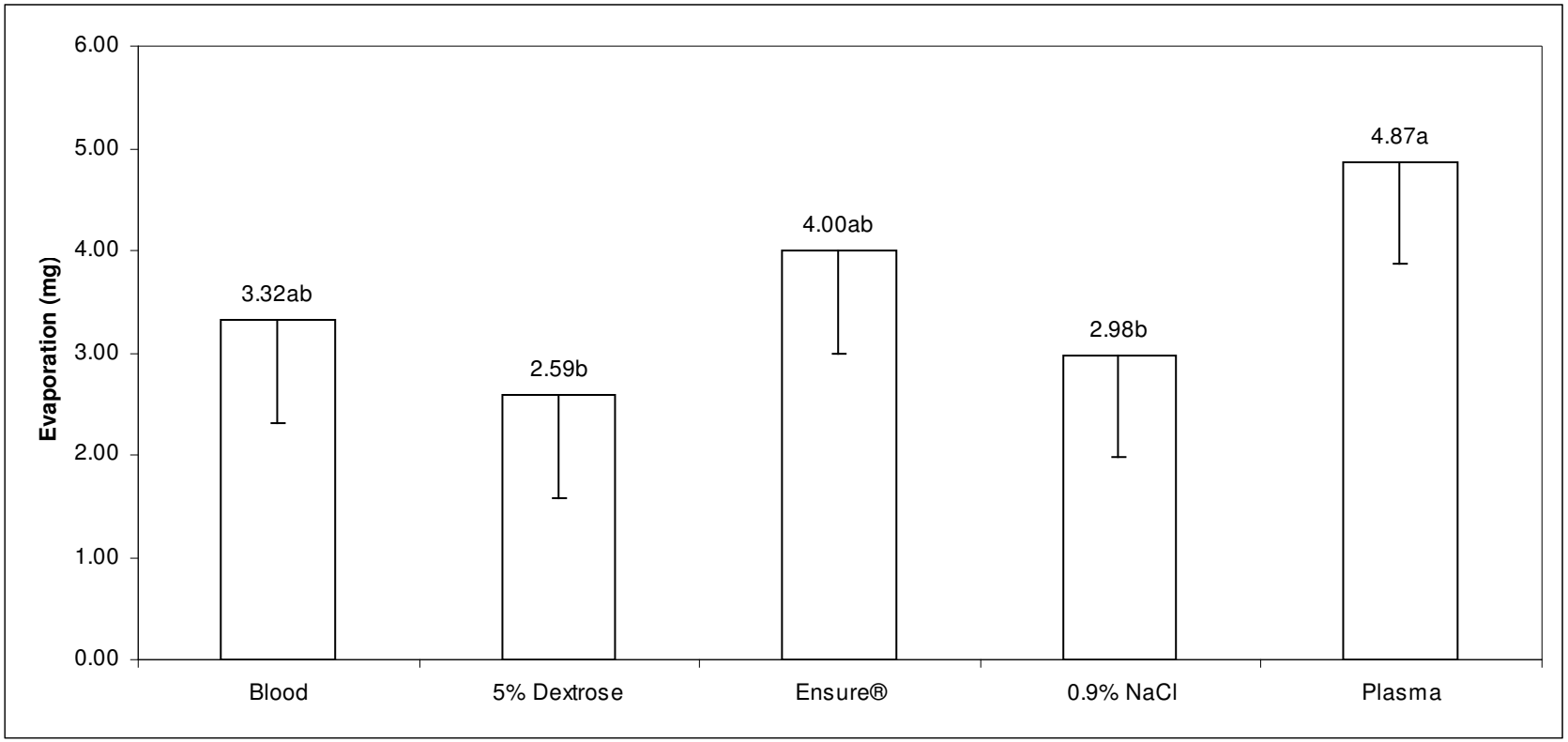

Figure 4-3. Mean evaporation (choice test) of fluids used in hospital environments for patient-care (μg) (24 hours). Data was collected from the control arenas: evaporation was calculated by subtracting final mass of fluid remaining at the end of the 24 h test period from the amount of fluid initially provided. Means followed by same letter are not significantly different by Tukey's HSD-test ($P = 0.05$; SAS Institute 2000).

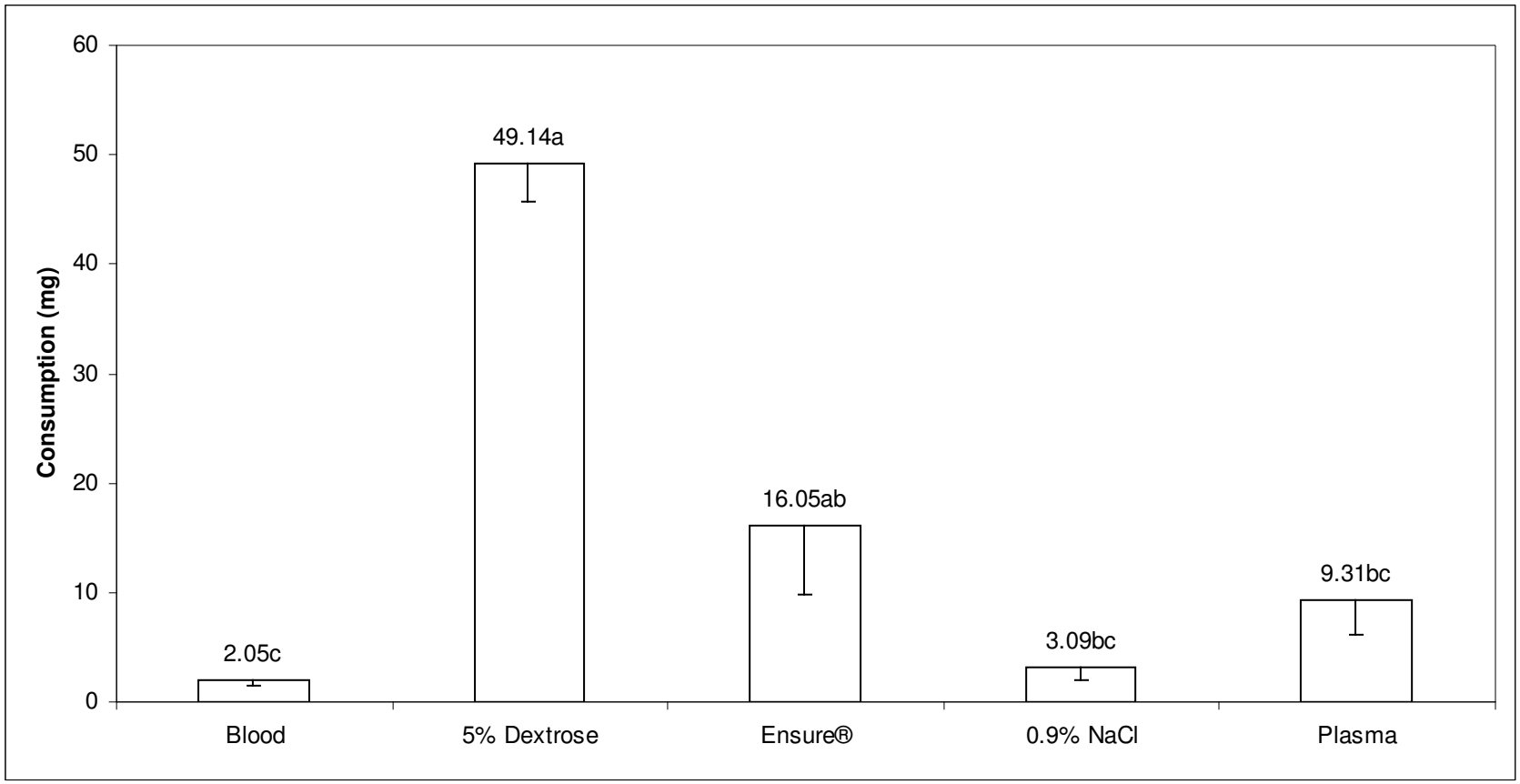

Figure 4-4. Mean (choice test) consumption (mg) for tested fluids used during test period (24 h) for starved (3 d) pharaoh ant colonies. Means followed by same letter are not significantly different by Tukey's HSD-test ($P = 0.05$; SAS Institute 2000).

CHAPTER 5
NO-CHOICE DEHYDRATED FLUID TEST

Introduction

Hospitals, nursing homes and other medical treatment facilities are often infested with Pharaoh ants (Green et al. 1954, Edwards and Baker 1981a, Eichler 1990), a tropical pest species that has worldwide distribution (Hölldobler and Wilson 1990, Fowler et al. 1994). Pharaoh ants have been discovered in sensitive patient-care areas, such as burn units where they were observed feeding on open wounds (Weidner 1982), in neonatal units where they were observed feeding upon the intact skin of newborns (Steinbrink 1978) as cited by Eichler (1990). Burn victims and newborns typically lie on beds containing sheets, and are provided with various fluids to meet hydration and nutritional needs. Fluids can spill and desiccate on sheets and might attract Pharaoh ants to the patient.

Consumption and preference tests were performed for 5 patient-care fluids used in hospitals to determine if Pharaoh ants actually consumed each fluid (Chapter 3) and to determine which fluids they preferred (Chapter 4) in their liquid state when given a choice. While they consumed all fluids, consumption of liquid dextrose was significantly higher than all other fluids and was the preferred fluid in both the no-choice and the choice tests.

These five fluids are typically administered directly to the patient via inserted tubes. Saline, 5% dextrose, red blood cells and serum are used to replace lost fluids (AABB 2002); saline and dextrose can also be injected peritoneally (into the

abdomen/stomach) (Wenzel et al. 2002). Ensure™ dietary supplement is unique among these five fluids since it is administered solely through injection into the patient's stomach (Wenzel et al. 2002). Regardless of the method used to provide these fluids to patients, any of these fluids might easily spill onto the patient bed and solidify upon the bed-sheets.

It was determined that these five fluids needed to be provided to the Pharaoh ants in their desiccated state (i.e., as solids) to determine if the ants demonstrated interest in spilled patient-care fluids in hospitals. The potential availability of these substances might be substantially greater in their dried form than in their liquid form, and could be a factor in attracting the ants to patient beds and to the patient. The objective of this experiment was to determine if Pharaoh ants would forage to desiccated patient-care fluids used in hospital using a no-choice test.

Materials and Methods

Ants

Pharaoh ant test colonies were extracted from large maintenance/parent colonies housed and reared at the Urban Entomology Laboratory, University of Florida, Gainesville, FL. See Appendix A for full details on maintenance of ants. Colonies consisted of 250 workers, 3 dealate queens and approximately 50 mg brood.

One hundred twenty-five workers were selected from cells to ensure presence of nurses and 125 workers were selected from outside cells to ensure presence of foragers (Haack 1987, Haack and Vinson 1990, Haack et al. 1995). Brood included eggs, all larval stages and pupae. See Appendix B for full details on selection of ants and preparation of experimental test colonies.

Foraging Arena and Nest

Foraging arenas (Fig. 5-1) consisted of clear plastic boxes (27 cm by 19 cm by 10 cm deep; Catalogue No. 195C, Pioneer Plastics, Dixon KY) which were coated with Fluon™ (Catalogue No. AD1, Asahi Glass Fluoropolymers, Downington, PA) on interior sides to prevent ant escape. One nest cell was placed within one corner of each arena. Cells consisted of sterile, covered plastic Petri dishes (50 mm diameter by 15 mm depth; Falcon® Model No. 351007, Becton Dickinson and Co., Franklin Lakes NJ) filled halfway with Castone dental plaster (Model No. 99045, Dentsply International, York PA). One entrance hole (~5 mm diameter) was melted into the upper side wall of the Petri dish base via insertion of the metal tip of a hot glue gun (*sans* glue) just above the level of the hardened plaster. The Petri dish lid was placed on top of the base and the entire nesting cell was placed into the foraging arena with the entrance hole facing the opposite diagonal corner of the arena.

Water was provided *ad libitum* in a 15 ml vial (Model No. 55-9, Thornton Plastics, Salt Lake City UT) with three large cotton balls inserted into the top to prevent leakage and to provide easy access for the ants. The water vial was placed in the opposite diagonal corner of the test unit arena across from the cell.

Hospital Sheets

Hospital sheets (Forest General Hospital, Newberry Road, Gainesville, FL) were washed in extremely hot temperatures and underwent a rigorous sterilization process (John Bennet, personal communication). Freshly laundered hospital sheets were obtained

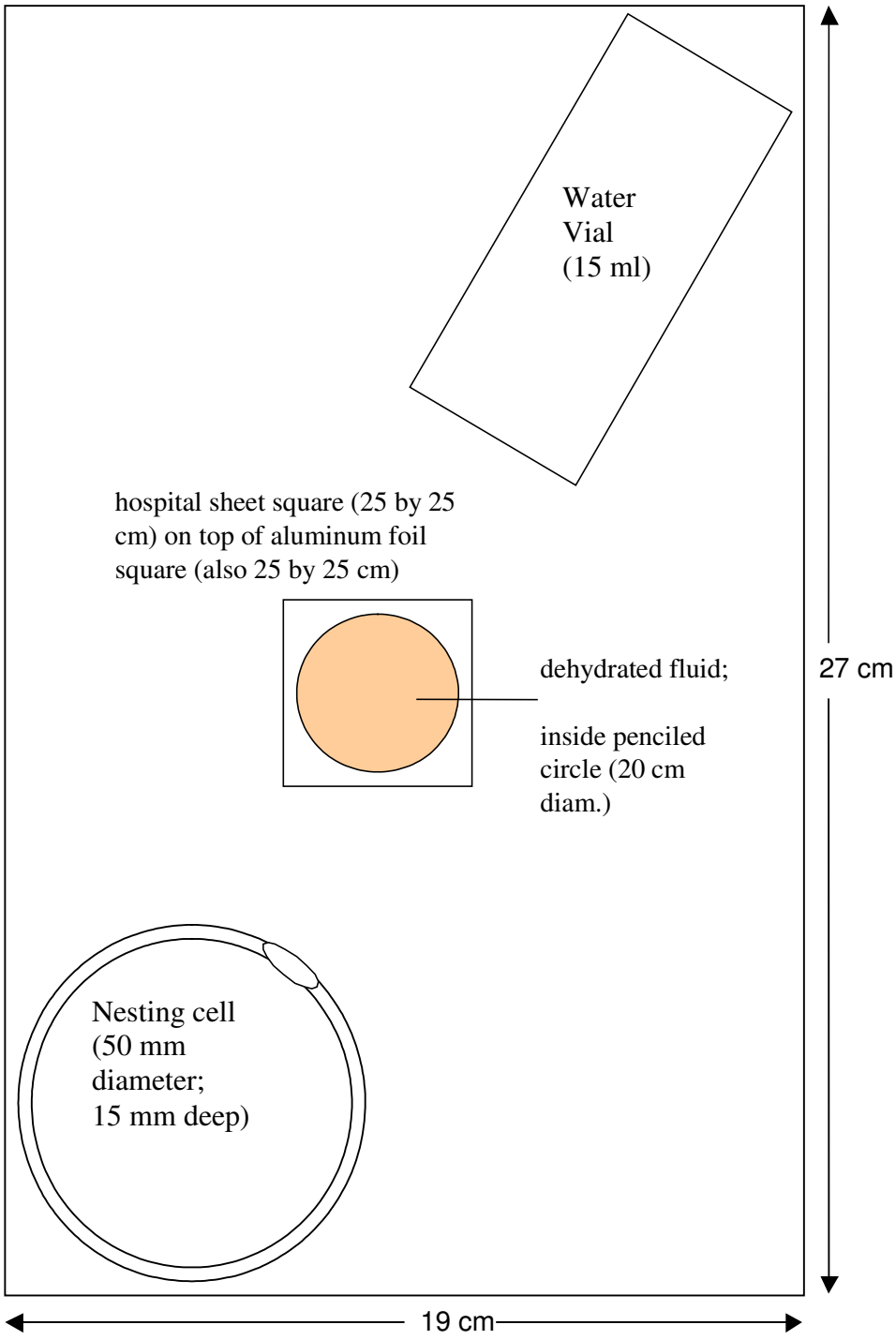

Figure 5-1. Arenas for dehydrated liquid food no-choice experiment. Arenas contained 125 inside workers, 125 outside workers, 3 dealate queens, and ~50 mg brood (all stages). Fluids drops placed on hospital sheets desiccated for 4 d and placed in center of arena, resting on aluminum foil. Fluids placed within penciled circle (20 cm diameter).

from a local hospital and were cut into small square (25 by 25 cm). Circles (20 by 20 cm) were penciled onto hospital sheets and were centered so that a 2.50 cm border existed between the penciled circle and the exterior border of the sheet. Pencil notations were made along the edge of the hospital sheet, outside of the circled border to identify the fluid applied to a sheet.

Heavy-duty aluminum foil was cut into small squares (25 by 25 cm) and placed under the hospital sheets. Each square of aluminum foil with its respective hospital sheet square was placed into a large (40 by 52 by 12 cm deep) plastic tray (Model No. 400-5N, Panel Controls, Greenville, SC) and suspended ~0.3 m above a portable room heater in preparation for fluid application and dehydration.

Dehydration of Fluids

Fluids used in this experiment consisted of tap water and 5 liquid patient-care fluids: human blood cells, human plasma, 5% dextrose and 0.9% NaCl. Each fluid was placed in a sterile test tube. Clean, dry plastic 15 ml disposable pipettes were used to withdraw fluid and place a drop of ~1 ml volume in the center of the 25 by 25 cm hospital sheet, within the penciled circle's 20 cm boundaries. A separate pipette was used for each fluid, and fresh pipettes were used each time. No mixing of pipettes occurred. Drops were left to air-dry for 1-2 h until visual observation indicated thorough desiccation. Drops were added in this manner until a total of 5 drops were placed on each hospital sheet. After the final drop dried, the large tray was placed on a lower wall-rack in the experimental laboratory in away from the light, and remained there for 4 d until used in the experimental test. The tray was not covered.

Room temperature was maintained at 26.7° C – 28.9° C and relative humidity fluctuated between 40-56%. Fluids were permitted to dehydrate during the same period

that ants were starved. Standardized Pharaoh ant colonies were starved (only water provided) for 72 h after being placed in the arenas. The hospital sheet square containing the dried substance was placed in the center of the test unit, halfway between the nest cell and the water. Each arena was covered with a clear plastic lid to maintain humidity and temperature within arena.

Experimental Design

The experiment was designed as a randomized complete block, blocking on colony. Each block consisted of Pharaoh ant colonies (125 inside workers, 125 outside workers, 3 dealate queens and ~50 mg brood) obtained from the same parent colony and randomly assigned to each treatment (dehydrated fluid type).

Ants were selected from 6 different parent colonies. A single fluid was offered to each test colony. Control arenas located adjacent to test arenas were identical except that they lacked ants.

Timed Observations

Observations of foraging behavior were noted upon immediate placement of fluids into arena, and the number of ants that appeared to be feeding on the dried fluids began 15 min after initial exposure and continued at 15 min intervals for 2 h. Ants were considered to be feeding if they were standing stationary and assumed a feeding posture with antennae and/or palps touching dried fluids that were contained within the circled portion of the hospital sheet, regardless of how much of their body was within the circle.

Ants feeding on fluid that spilled beyond the penciled circle were not counted. Similarly, ants engaging in trophallaxis were not counted, even if this activity occurred in the center of the circle. Ants that were in a resting posture within the circle were also not counted. Ants that were walking or otherwise actively moving during the timed

observation intervals were not counted, even if antennae were in contact with dehydrated fluids.

Statistical Analysis

Four analyses were done using analysis of variance (ANOVA) with means separated using Tukey's HSD (SAS Institute 2000). First, counts of feeding ants over all dried fluids among the 15 min intervals were compared. Second, the total number of ants feeding on each dried fluid for the entire 2 h test period was compared.

Third, for each time interval, the mean numbers of feeding ants were compared among fluid types. Finally, for each fluid, the mean numbers of ants feeding were compared among each of the 15 min intervals.

Results

Usually 1 or 2 ants were foraging in the arenas during placement of dehydrated fluids into arenas. Foragers in arenas containing Ensure and dextrose exhibited an immediate change in behavior ─ they rapidly increased their walking speed and began waving antennae in front and above their heads in an excited manner. They took fast running steps in short bursts towards the dehydrated fluid, and located the fluid within a few seconds; recruitment of additional ants in arenas containing Ensure and dextrose frequently occurred in less than 5 min. In arenas with dehydrated dextrose, foragers exhibited a tendency to feed for several minutes before recruiting other ants. In arenas with dehydrated Ensure, initial foragers tended to engage in extensive antennal tapping of dehydrated fluid while rapidly encircling the entire hospital sheet; after completely circling the fluid they rapidly ran to the nesting cell. Shortly afterwards, several ants were observed proceeding in a hurried manner towards the Ensure, where most engaged in feeding behavior for several minutes. A few returned to the nest, again resulting

immediately in an increased number of foragers emerging from the nest. Ant behavior in arenas in which hospital sheets soaked with blood cells, plasma, saline or water did not elicit any increased foraging activity or immediate recruitment behavior. Foragers in these arenas continued to walk around the arena in a slow, unhurried manner and did not seem to respond to dehydrated blood cells, plasma, saline or water as a food source. Occasionally, a forager was observed feeding on blood cells, plasma, or saline, but no foragers were observed on sheets that had been soaked with water. Foragers did not exhibit the same type of excited behavior in these arenas as was observed in arenas with high-sugar dehydrated fluids. Even after feeding for several minutes, ants were not observed running back to the nest, and no mass recruitment was observed in arenas lacking sugar.

Peak feeding of all fluids simultaneously occurred at 45 min and steadily decreased afterwards. However, ant counts were not significantly different amongst time intervals ($F = 0.97$, df =7, 1144; $P = 0.4499$) (Fig. 5-2). The number of Pharaoh ants feeding upon Ensure® (13.89 $\pm$ 0.53) was significantly higher than on all other dehydrated fluids. Feeding upon 5% dextrose was significantly higher than on plasma, blood cells and saline ($F = 617.75$, df = 5, 1146; $P < 0.0001$) (Fig. 5-3). Numbers of ants feeding upon Ensure® was significantly higher at each time interval than on all other fluids (Fig. 5-4 through 5-11). There was no significant difference in numbers of ants feeding on all other fluids per time interval. Only Ensure® and dextrose had significant feeding among time intervals during the 2 h experiment. Feeding of Ensure peaked at 45 min (18.0 $\pm$ 2.0 ants) (Fig. 5-12) and feeding of dextrose also peaked at 45 min (2.4 $\pm$ 0.4 ants) (Fig

5-13). Feeding on the saline, blood cells, and plasma were virtually non-existent as mean counts at any time interval was never higher than 0.2 ants (Fig. 5-14, 5-15, 5-16).

Discussion

Pharaoh ants were starved for 72 h in this no-choice solid food test – this was 24 h longer than starvation period of 48 h used in my liquid consumption tests (Chapters 3, 4). In contrast, a 72 h starvation period resulted in observable foraging behavior for nearly all test arenas. Starvation periods >72 h were not attempted because Pharaoh ant recruitment to carbohydrates can be very high after extensive starvation, due to a more rapid depletion of carbohydrate stores (Nation 2003). Haack (1987) reported that 7 d starvation made it difficult to count numbers of foraging Pharaoh ants because they overwhelmed high-sugar foods upon initial exposure. Haack et al. (1995) reported that 2 to 3 d starvation was sufficient to deplete protein stores enough to elicit strong foraging behavior for proteins. Ensure® was the only fluid with significant consumption throughout the experiment, and it was noteable that it was the only complex fluid provided in this experiment. It has high concentrations of all three major nutrients, with 17% protein, 17% lipid, and 26% carbohydrate.

The number of ants recorded feeding at both Ensure® and dextrose peaked at the 45 min time interval, although is was not significantly different from ant counts of most other time intervals. These data differ slightly from that collected by Haack (1987) who reported that 93% of workers fed within the first 30 min of food placement in the arena. The Ensure® and dextrose were the only fluids to have significant numbers of ants feeding upon them, and they shared a similar trait: high sugar content. Although the types of sugars presented differed and were present in their solid form, they elicited foraging and feeding behavior on a significant number of foraging ants. Foraging to

solid, dehydrated sugar here differs from the results of Haack et al. (1995) who observed that Pharaoh ants did not respond to solid sugar crystals as a food source. In fact, they observed the ants filling tiny cracks in the nesting cell walls with the sugar crystals. Since the solid sugars used by Haack et al. (1995) were manufactured and sold as table sugar, both enantiomers of glucose were probably present in those crystals. In contrast, the solid sugar present in my experiment was crystalline structures of D-glucose, the biologically active enantiomer which does not require energy for digestion, since it is passively absorbed through the midgut wall and stored primarily as glycogen (Nation 2003).

Although Pharaoh ants appeared to feed upon dextrose, they aggregated around and appeared to feed upon Ensure® at much higher levels. Ensure® was themost viscous fluid used in this experiment and was the only food that contained multiple nutrients. It took longer to desiccate than other fluids, and when finally dry, appeared as a brown-colored textured glob that had a three-dimensional texture. In contrast, all clear fluids were difficult to see. Repeated applications of dextrose were visible because they resulted in a noticeable stiffening of the hospital sheet, and often adhered to the sheet to the underlying aluminum foil. Plasma was observable because it left a straw-colored stain on the hospital sheet. Blood was easy to see, but it was difficult to apply to the sheet in controlled amounts since the blood cells were very sticky. Blood cells dried in globs that were relatively large in comparison to clear fluids, although blood cells and Ensure had almost the same consistency. Saline and water were nearly impossible to see visually although the application left a wet spot, so that application could be confirmed

immediately after application. Desiccation of saline resulted in a crispy hospital sheet while application of water did not appear to have any effect.

Significant feeding upon high-protein substances or water was not expected. Higher feeding of dextrose solids and lower feeding upon Ensure® were anticipated. In previous consumption studies, Pharaoh ants preferentially consumed 5% dextrose above all other fluids, so it was interesting that when dried, Pharaoh ants feeding was less than the Ensure dietary supplement.

Pharaoh ant recruitment to Ensure® occurred after 4 d desiccation, so this fluid still elicited foraging after several days. Thus if spilled, Ensure® may attract Pharaoh ants at least 4 d after drying out. The complete nutritional status of Ensure, available to them after 3 d of starvation eliminated the need for resource switching, since it contained all three major nutrients. Additionally, the workers may have used their 4-toothed (Ogata et al. 1998) mandibles to scrape and extract solid particles of the lumpy-textured Ensure residue. Adult workers do not eat solid food, although they can digest solids as larvae (Le Masne 1953) as cited in (Petti 1998). Instead, when consuming solutions or semi-solids containing liquids, adults filter the liquid portion through their buccal tube (Petti 1998) and store filtered solids in the infrabuccal pouch. The resulting compacted solids are later ejected as a pellet, by eversion of the infrabuccal pocket, after the ant's return to the nest containing the developing larvae (Petti 1998). Also, worker ants can pick up larger particles in their mandibles to transport those back to the larvae. *Solenopsis invicta* (Buren), another myrmicine species, has even demonstrated the use of tools to assist in food transportation back to the nest (Barber et al. 1989). High foraging numbers of ants at the desiccated Ensure fluid probably indicated that this desiccated fluid contained

nutrients needed by the larvae, or was suitable for larval consumption and subsequent larvae to worker trophallaxis. Due to its high sugar and lipid content, Ensure® fluid contained large energy reserves, despite its dehydrated state. Thus, it may have attracted the foragers with its high nutritional value, even though the food still had to be transported back to the nest and biologically modified by the larval trophallaxis.

Nutritional requirements of ant colonies may vary with colony size; natural Pharaoh ant colonies tend towards 1:1:1 ratios of workers:eggs:larvae (Peacock et al. 1955b). Although the experimental colonies used in this experiment were smaller than natural colonies discovered in human structures, such as that reported anecdotally by Bellevoye (1889), they were representative of the ratios, and were similar to experimental colony sizes used by Haack and Vinson (1990).

The attractiveness of dried Ensure® after at least 4 d portends possible problems by attracting ants to patients due to spillage on hospital linens. However, extended attractiveness potentially indicates that this fluid might be useful as part of bait matrix for Pharaoh ant control. Of course, further testing would be required to assess this potential relative to other bait attractants.

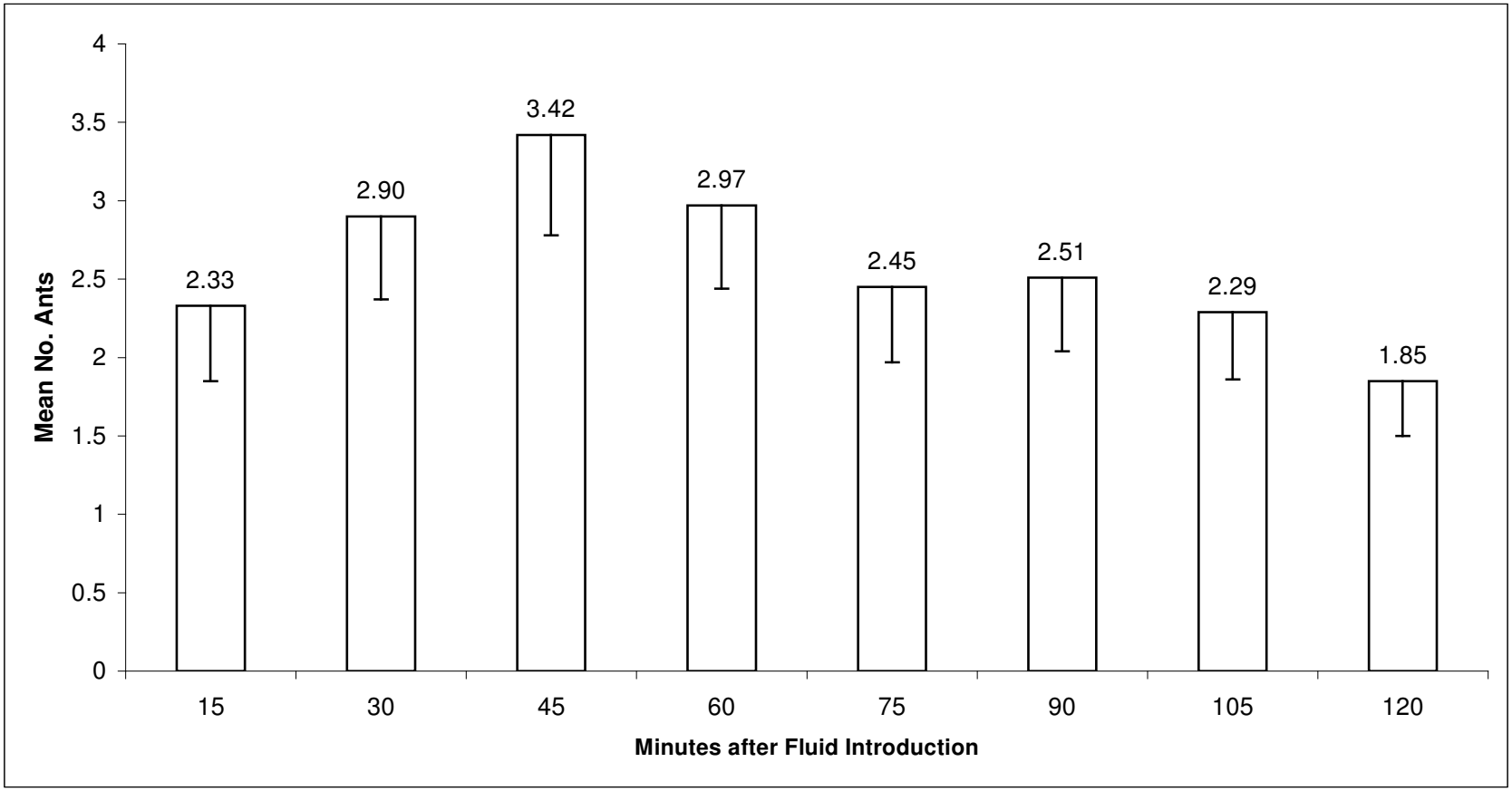

Figure 5-2. Mean number of ants feeding on all dehydrated fluids among 15 min intervals during 2 h test period. No significant differences among time intervals.

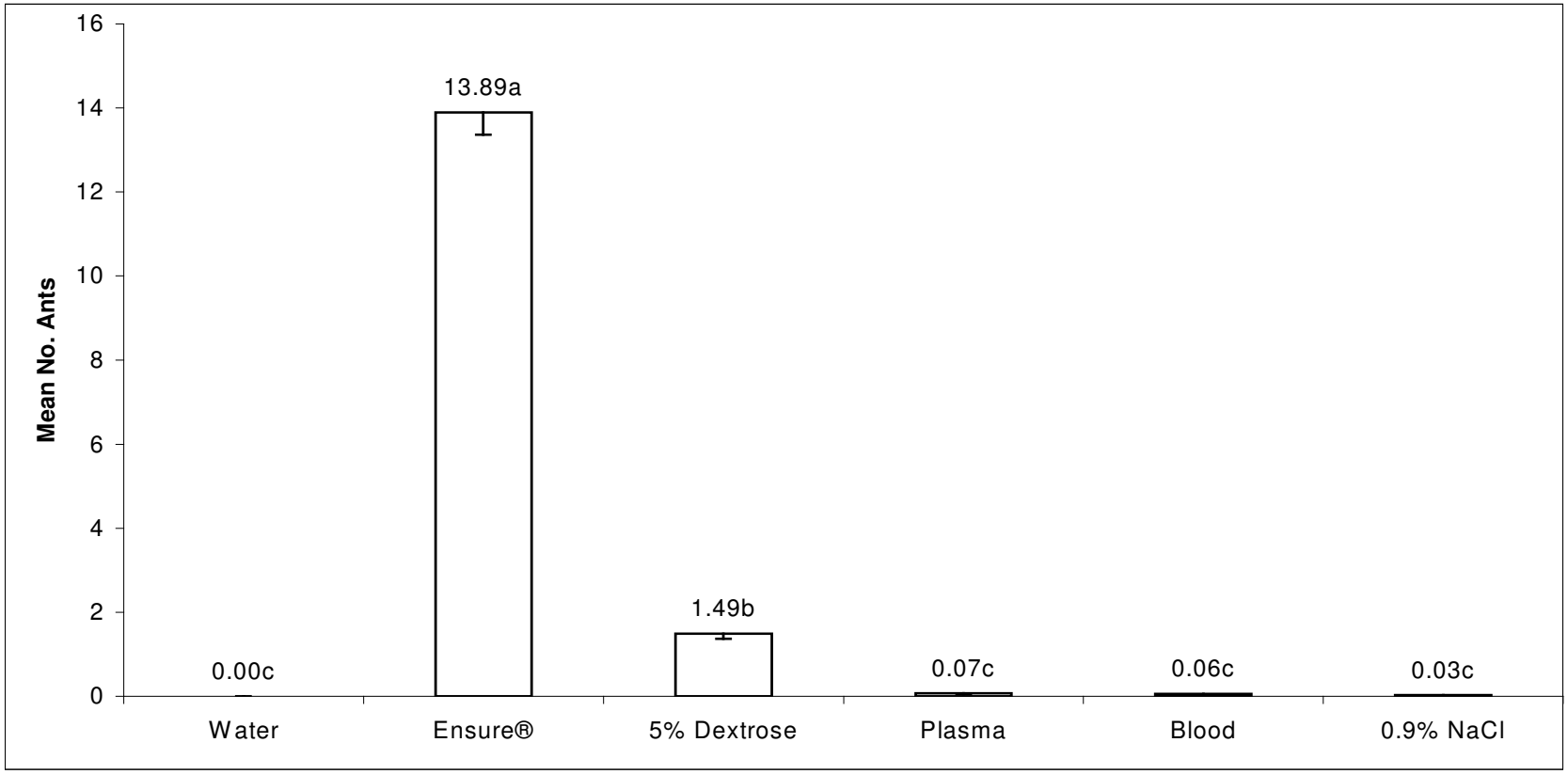

Figure 5-3. Mean number of foraging ants exhibiting feeding behavior at dehydrated fluids over entire 2 h test period. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

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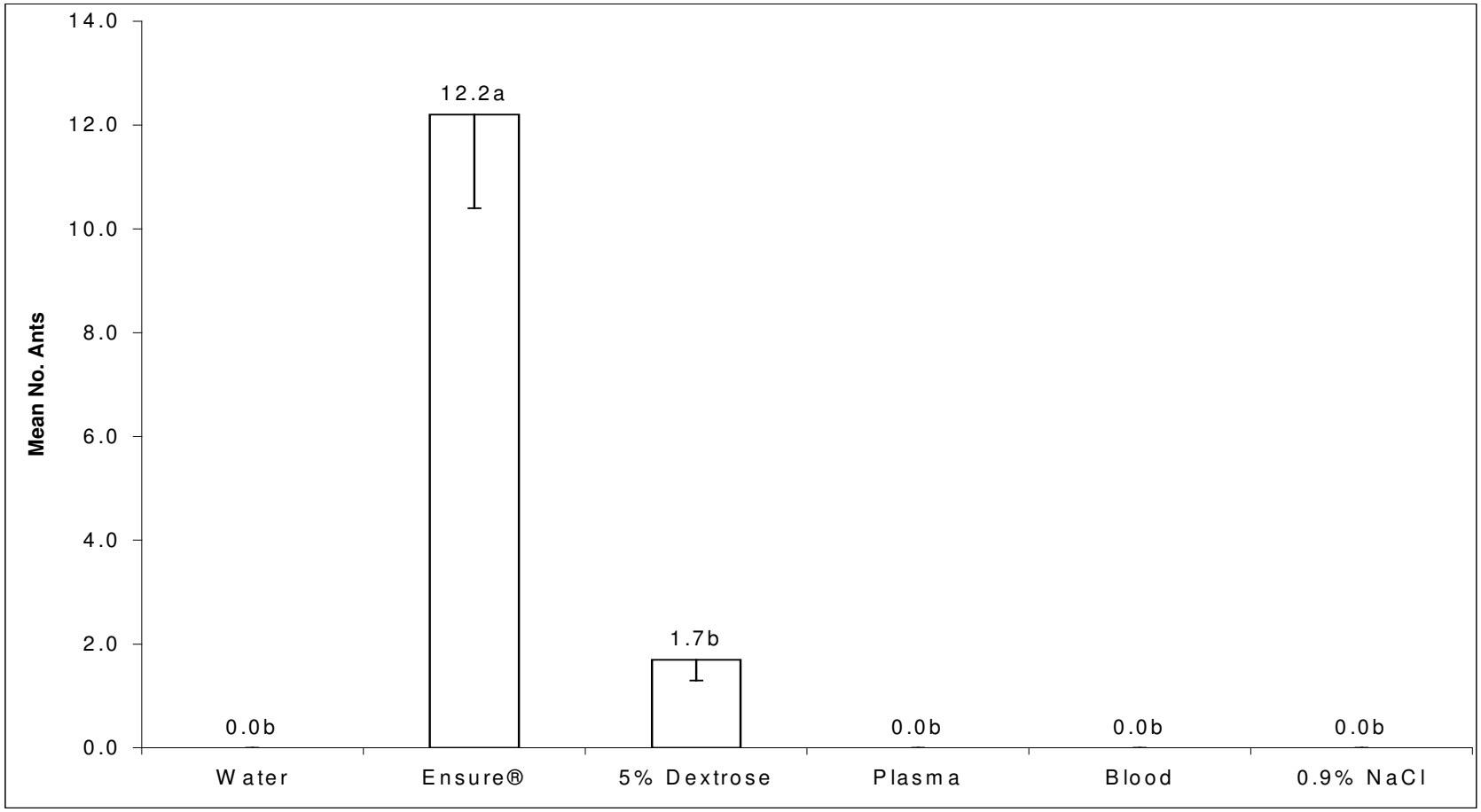

Figure 5-4. Mean number of ants feeding on at the 6 dried fluids at 15 min. time interval. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

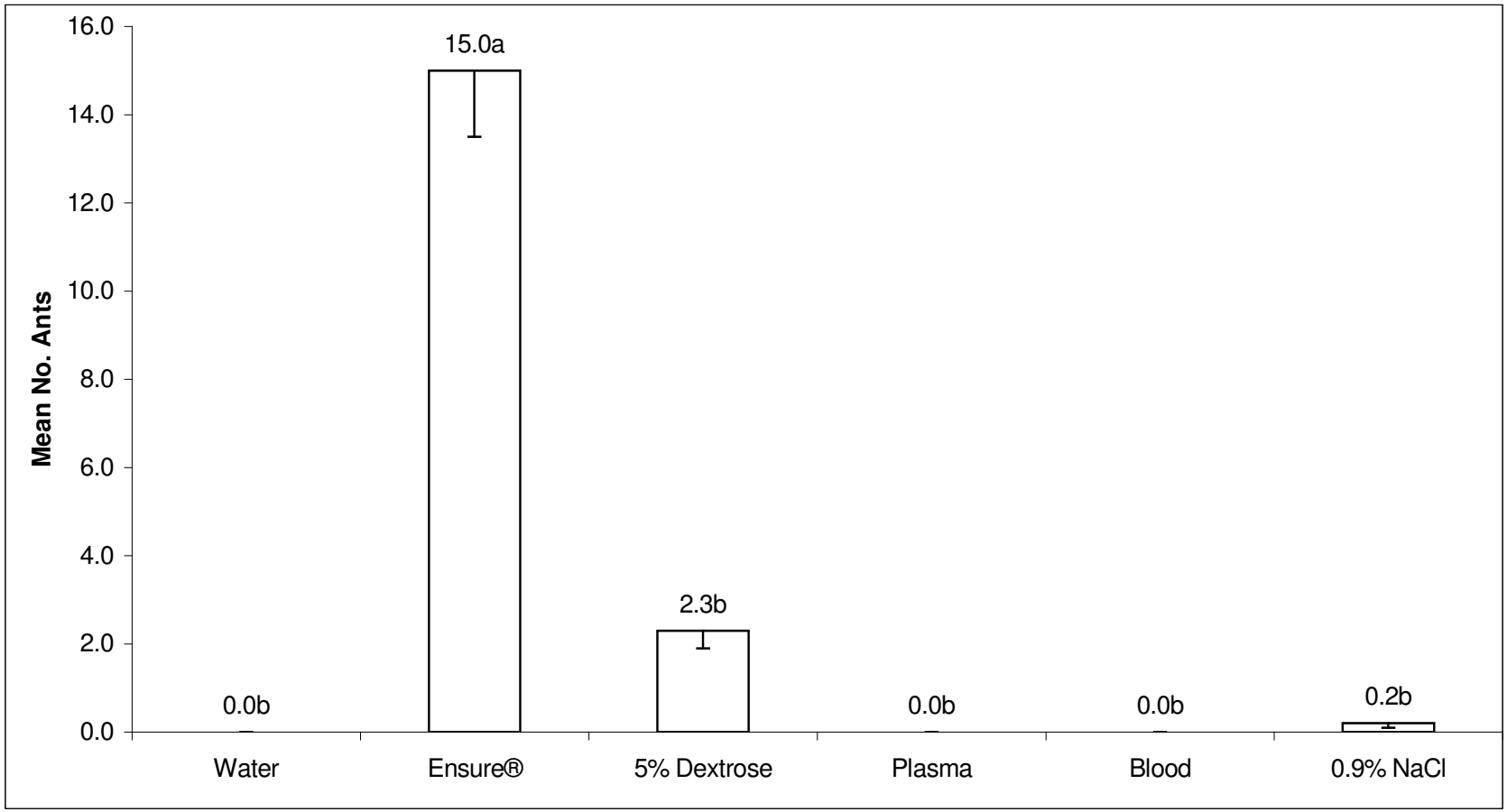

Figure 5-5. Mean number of ants feeding on at the 6 dried fluids at 30 min. time interval. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

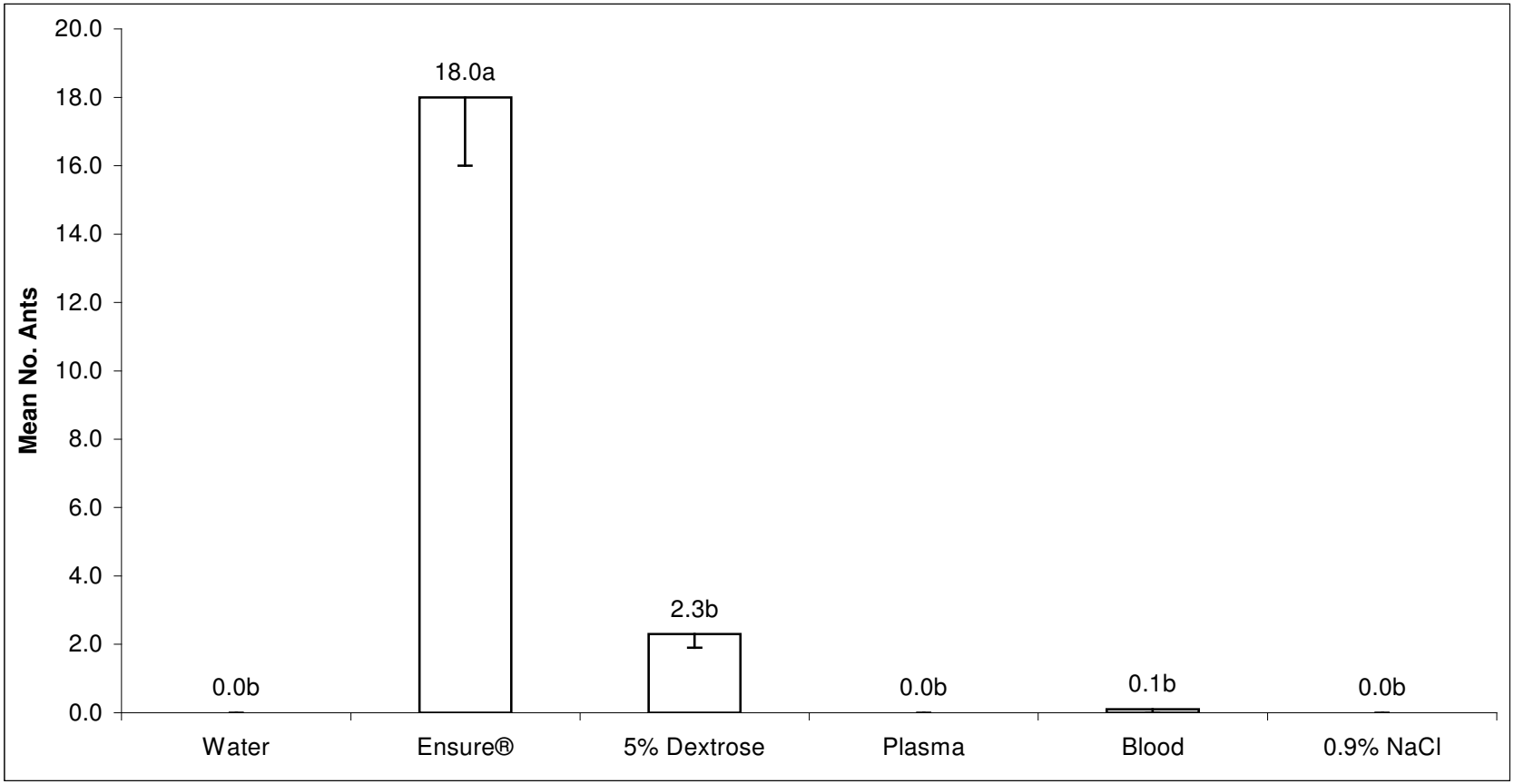

Figure 5-6. Mean number of ants feeding on at the 6 dried fluids at 45 min. time interval. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

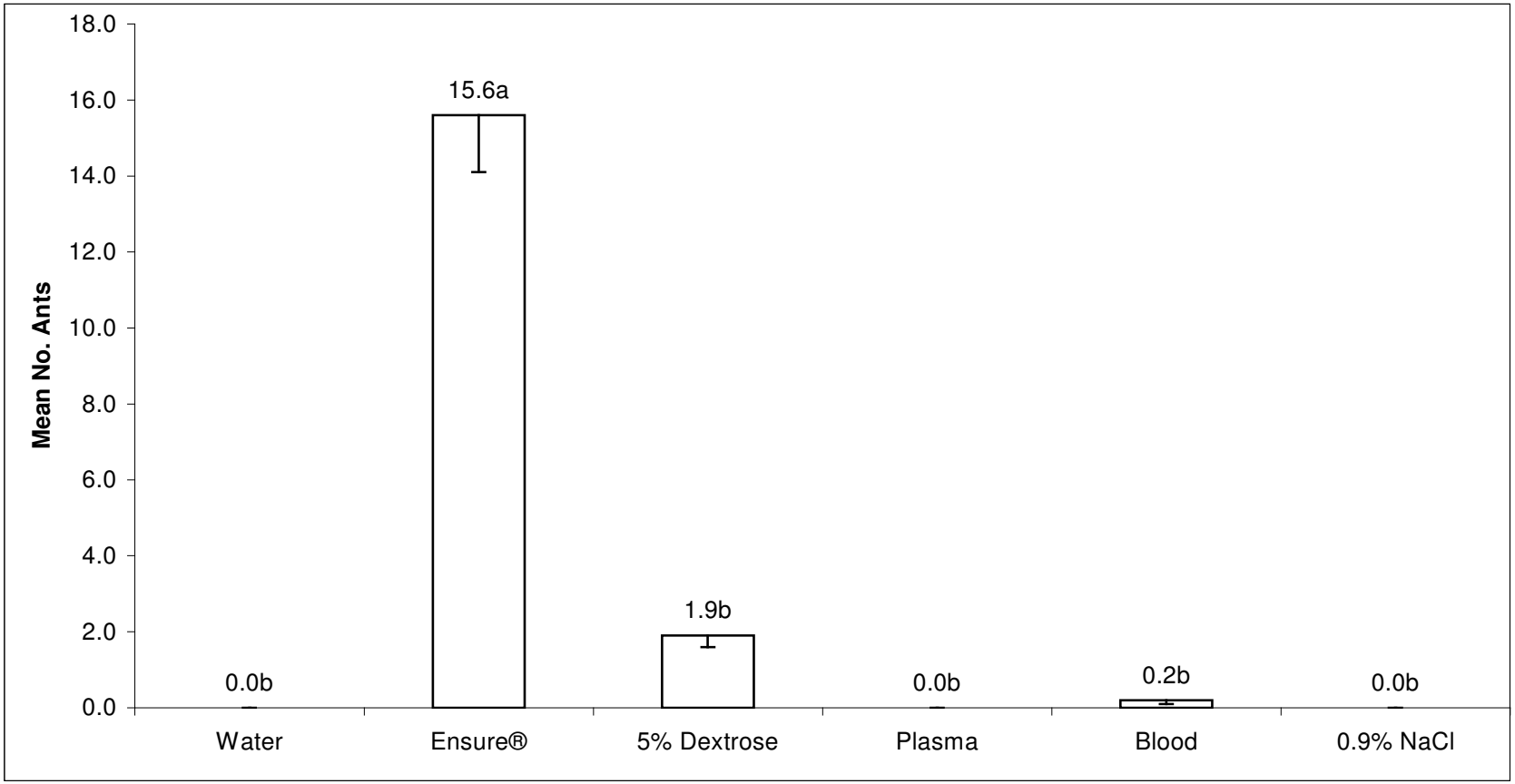

Figure 5-7. Mean number of ants feeding on at the 6 dried fluids at 60 min. time interval. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

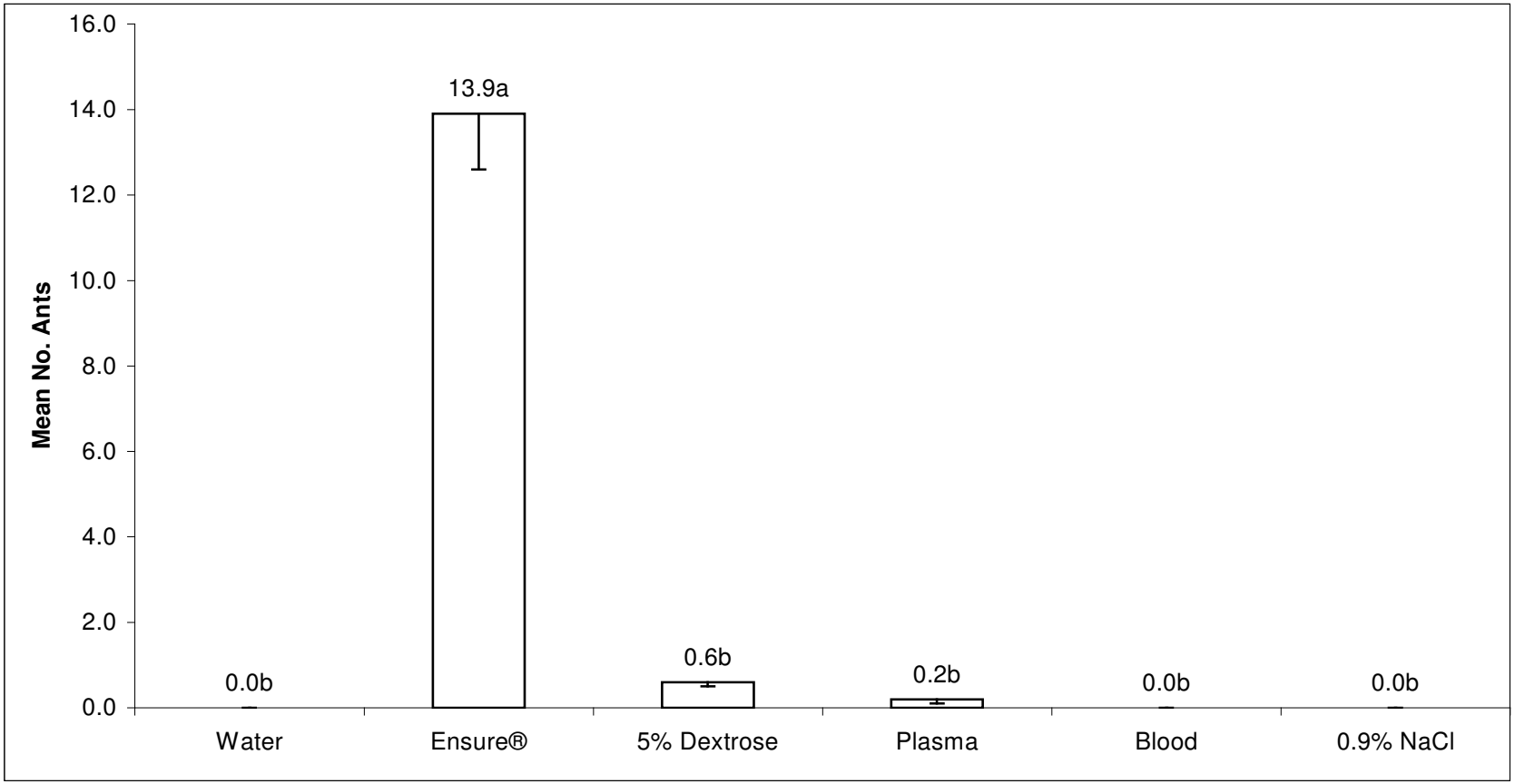

Figure 5-8. Mean number of ants feeding on at the 6 dried fluids at 75 min. time interval..Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

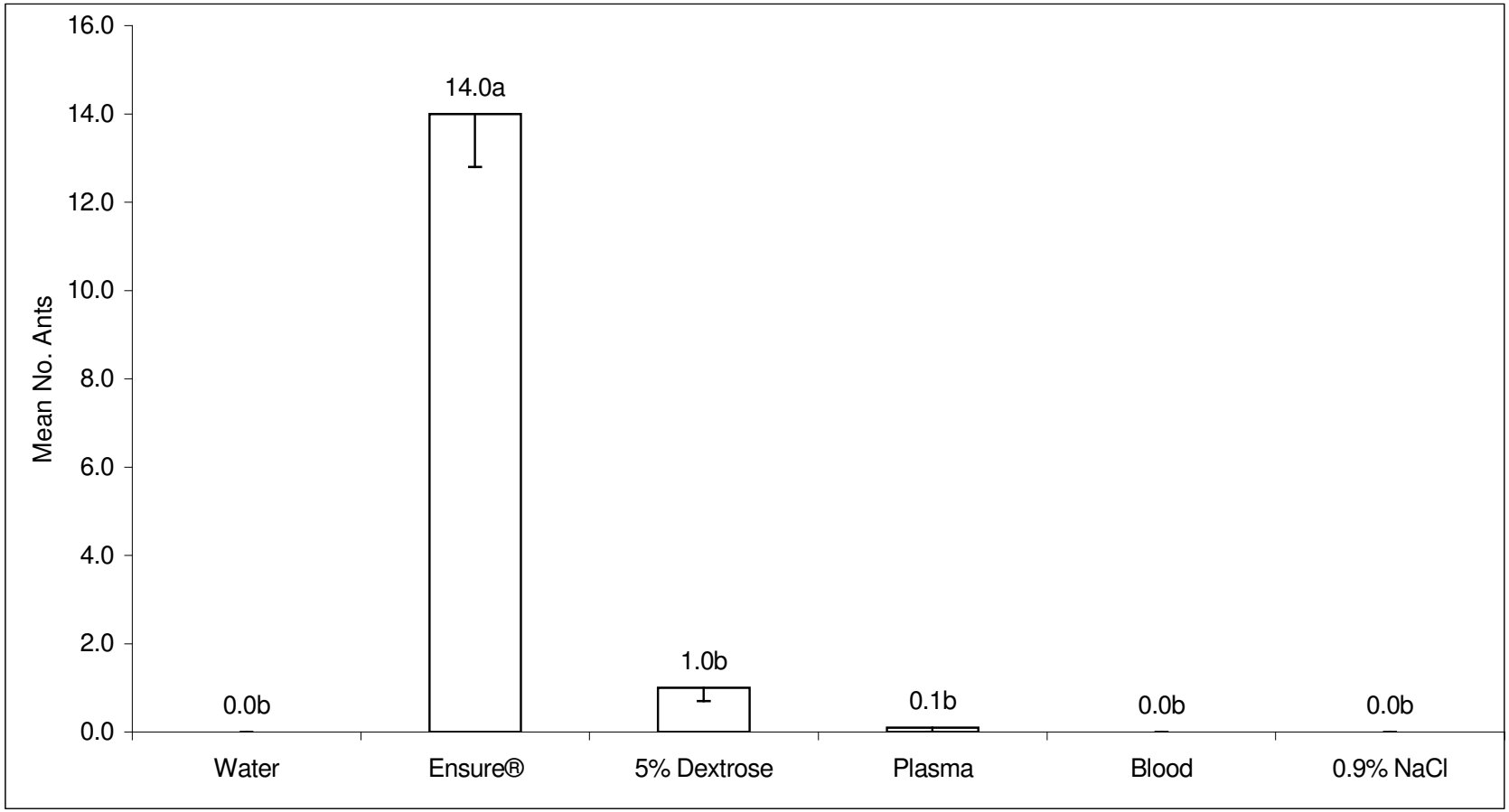

Figure 5-9. Mean number of ants feeding on at the 6 dried fluids at 90 min. time interval. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

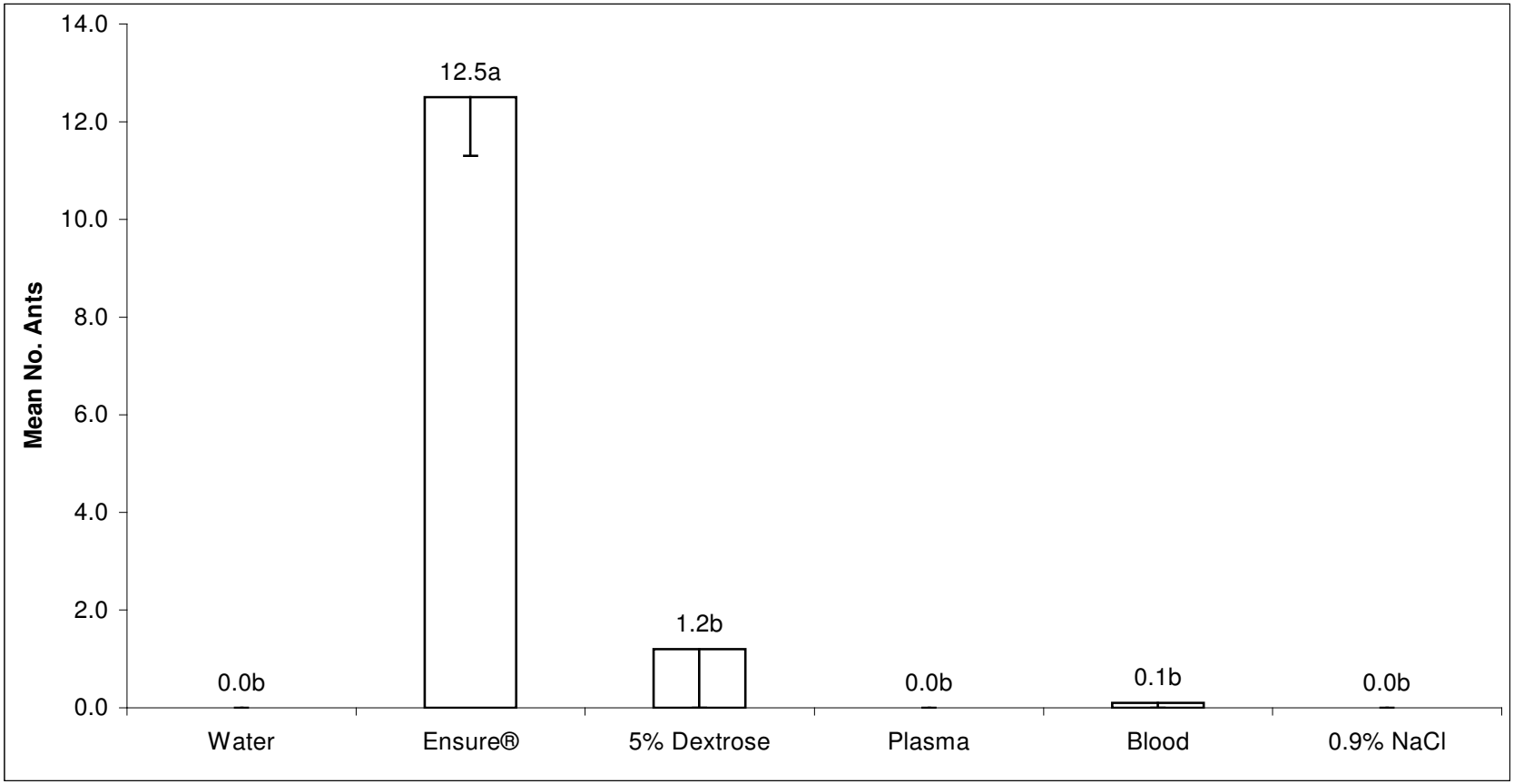

Figure 5-10. Mean number of ants feeding on at the 6 dried fluids at 105 min. time interval. Means followed by same letter are not significantly different (P <0.05) by ANOVA and Tukey's HSD test.

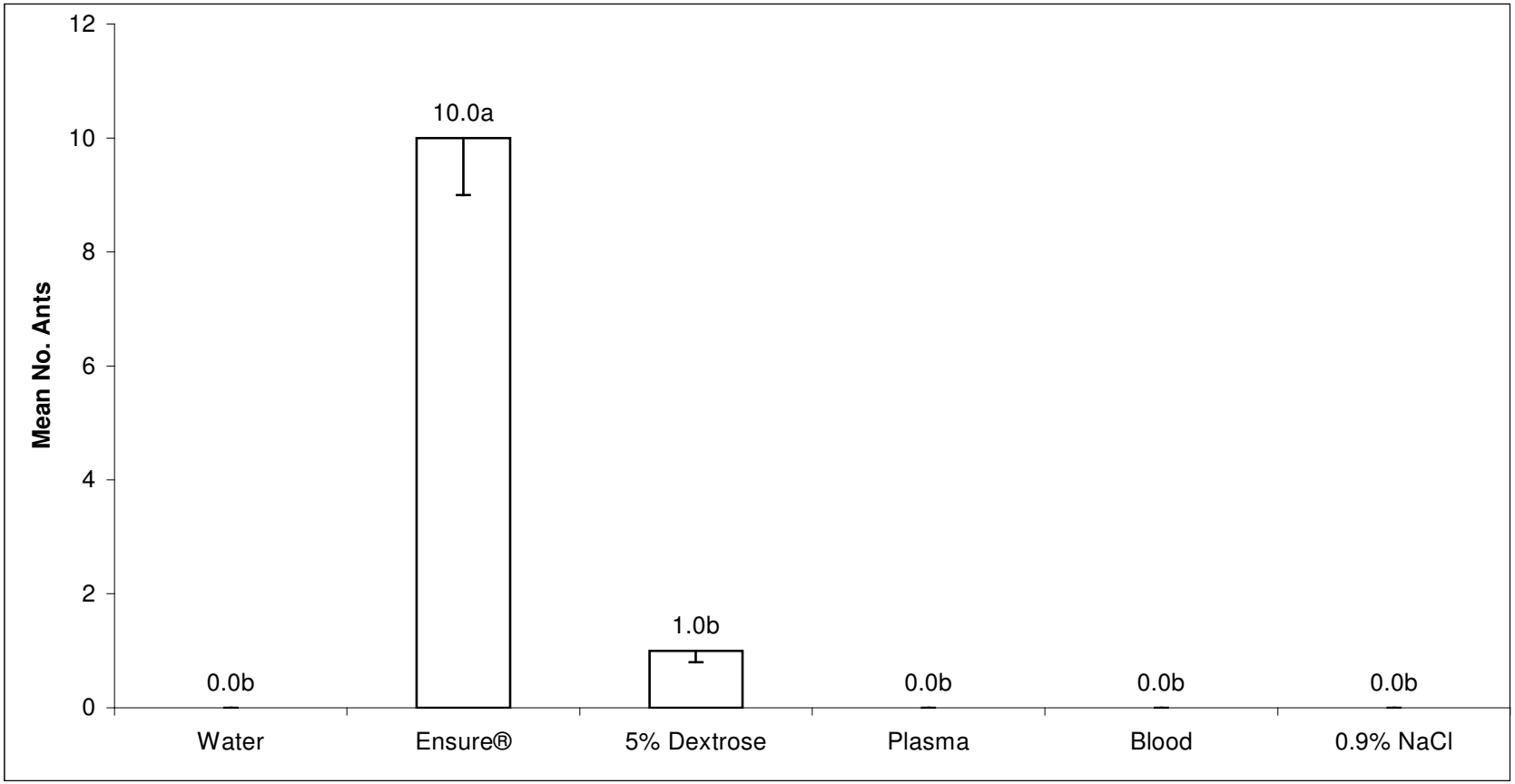

Figure 5-11. Mean number of ants feeding on at the 6 dried fluids at 120 min. time interval. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

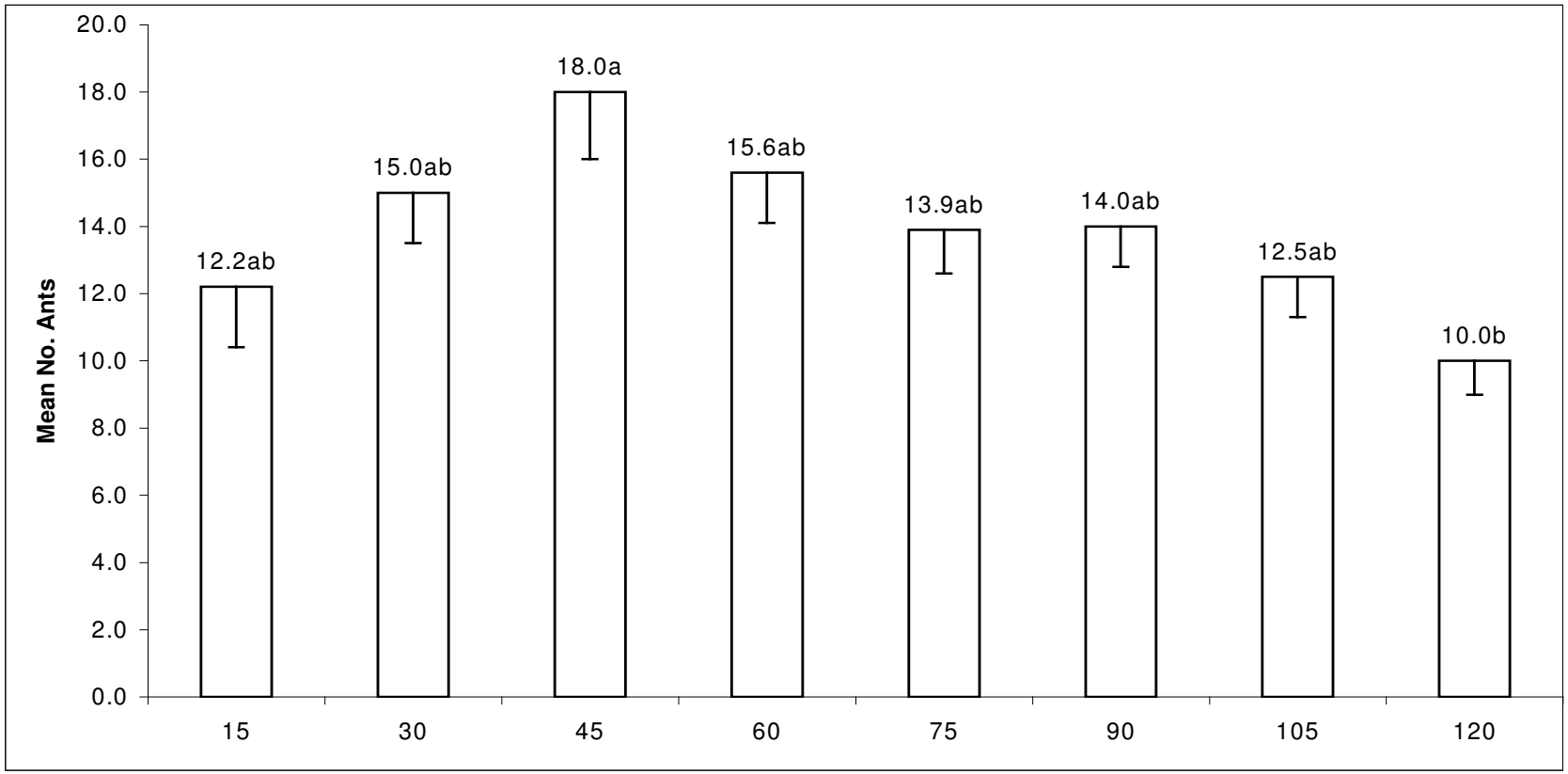

Figure 5-12. Mean number of ants feeding on Ensure® among 15 min intervals over the 2 h test period. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

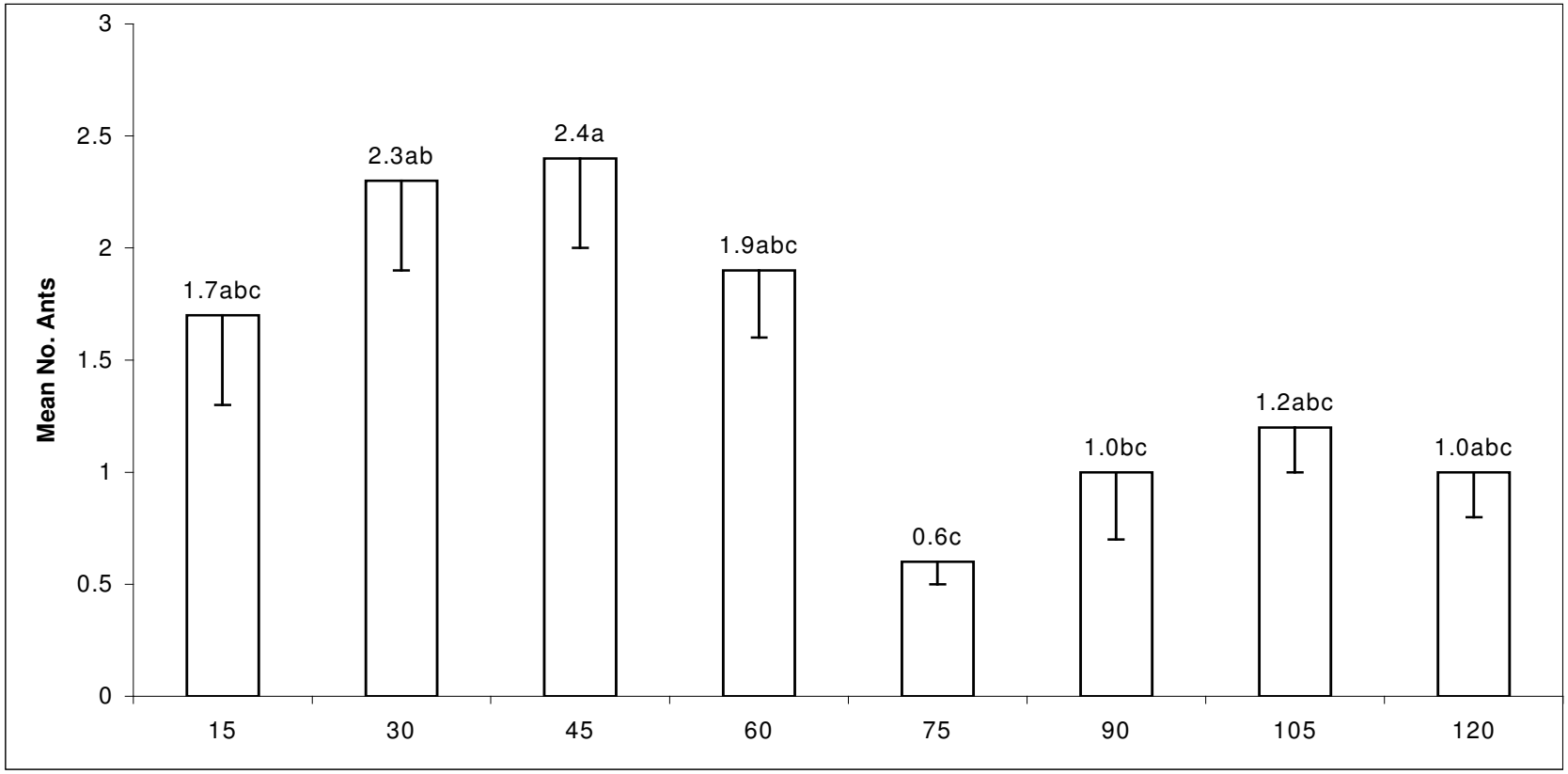

Figure 5-13. Mean number of ants feeding on 5% dextrose among 15 min intervals over the 2 h test period. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

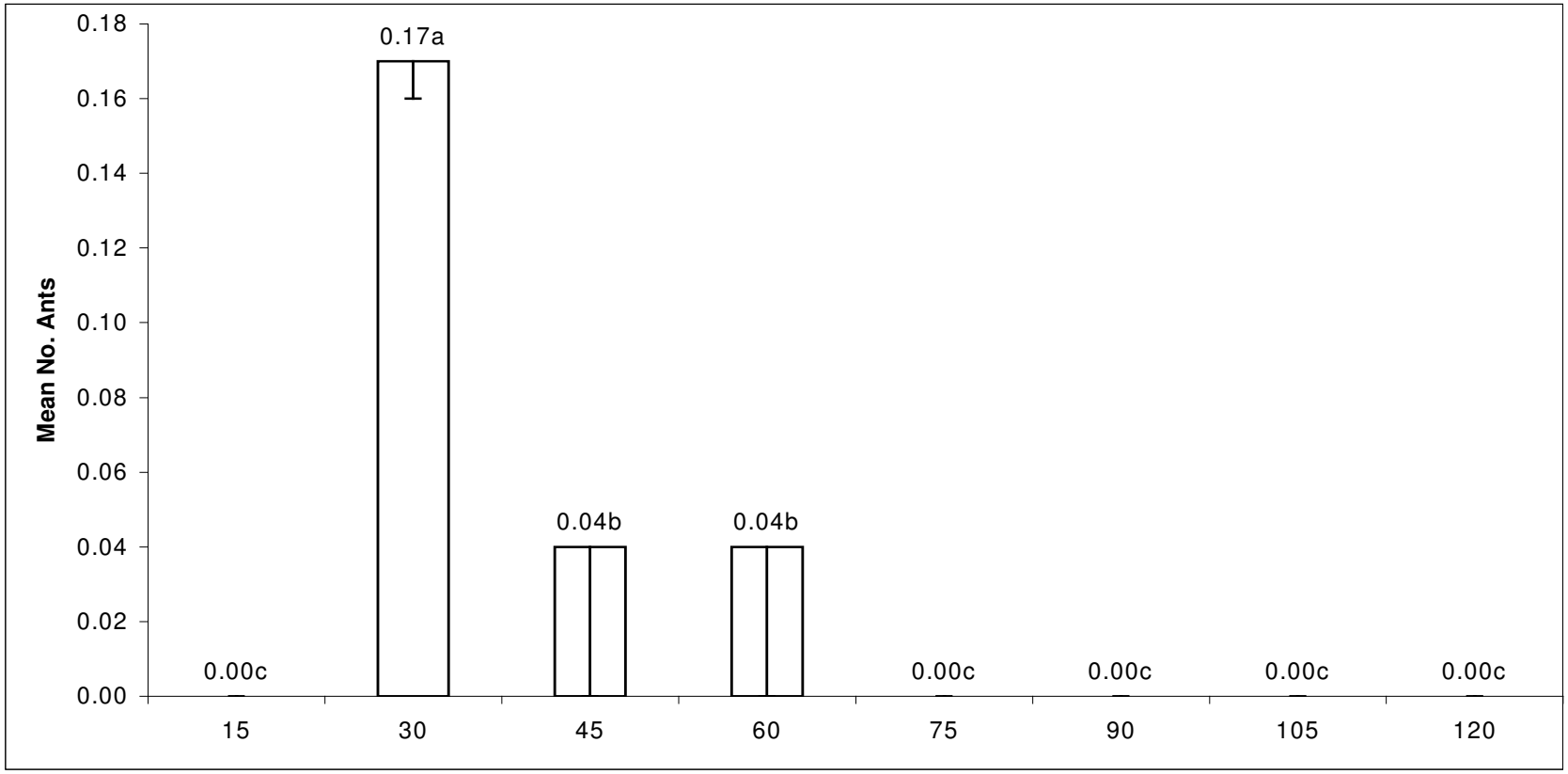

Figure 5-14 Mean number of ants feeding on saline among 15 min intervals over the 2 h test period. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

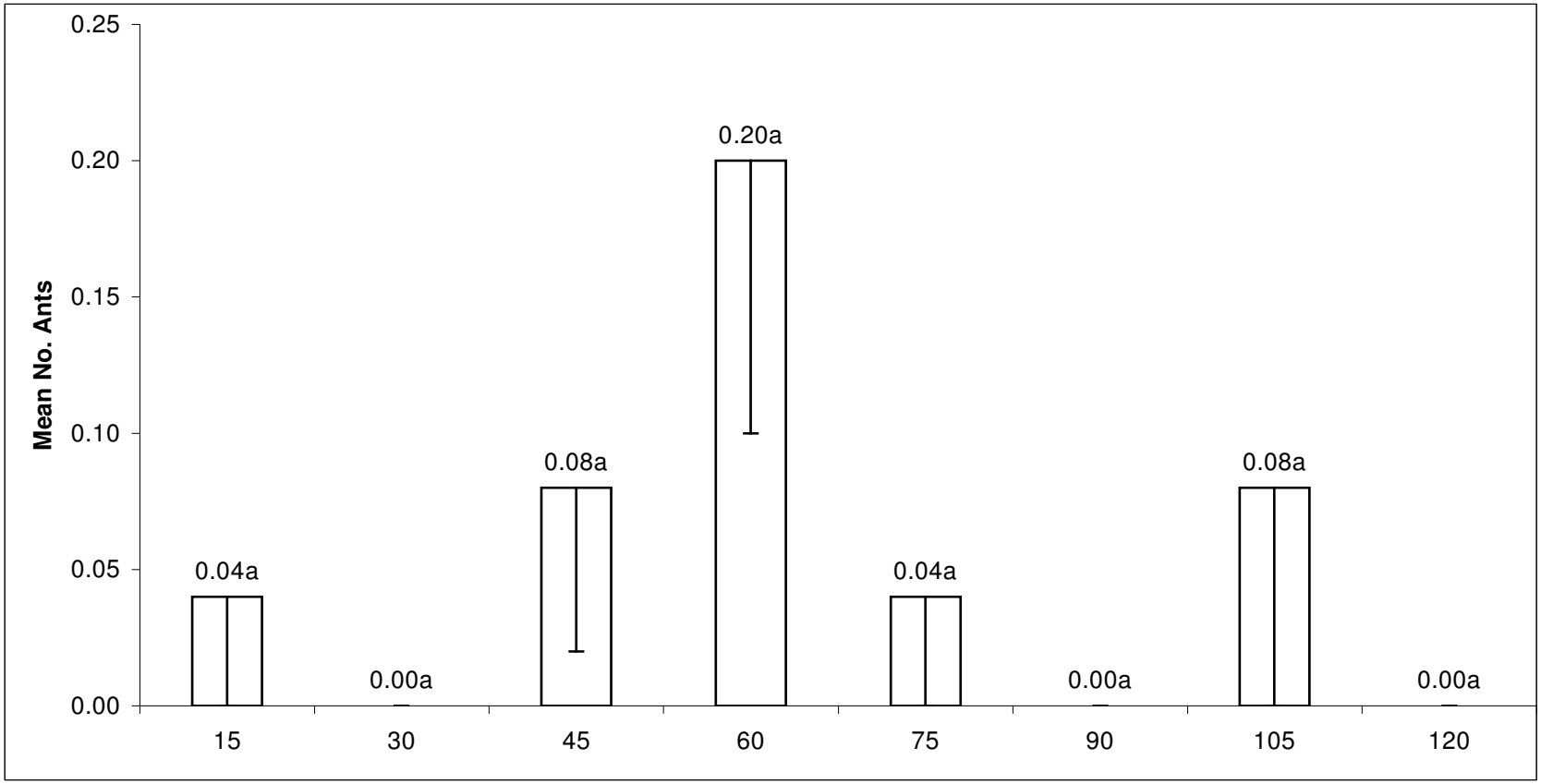

Figure 5-15. Mean number of ants feeding on blood cells among 15 min intervals over the 2 h test period. Means followed by same letter are not significantly different (P <0.05) by ANOVA and Tukey's HSD test.

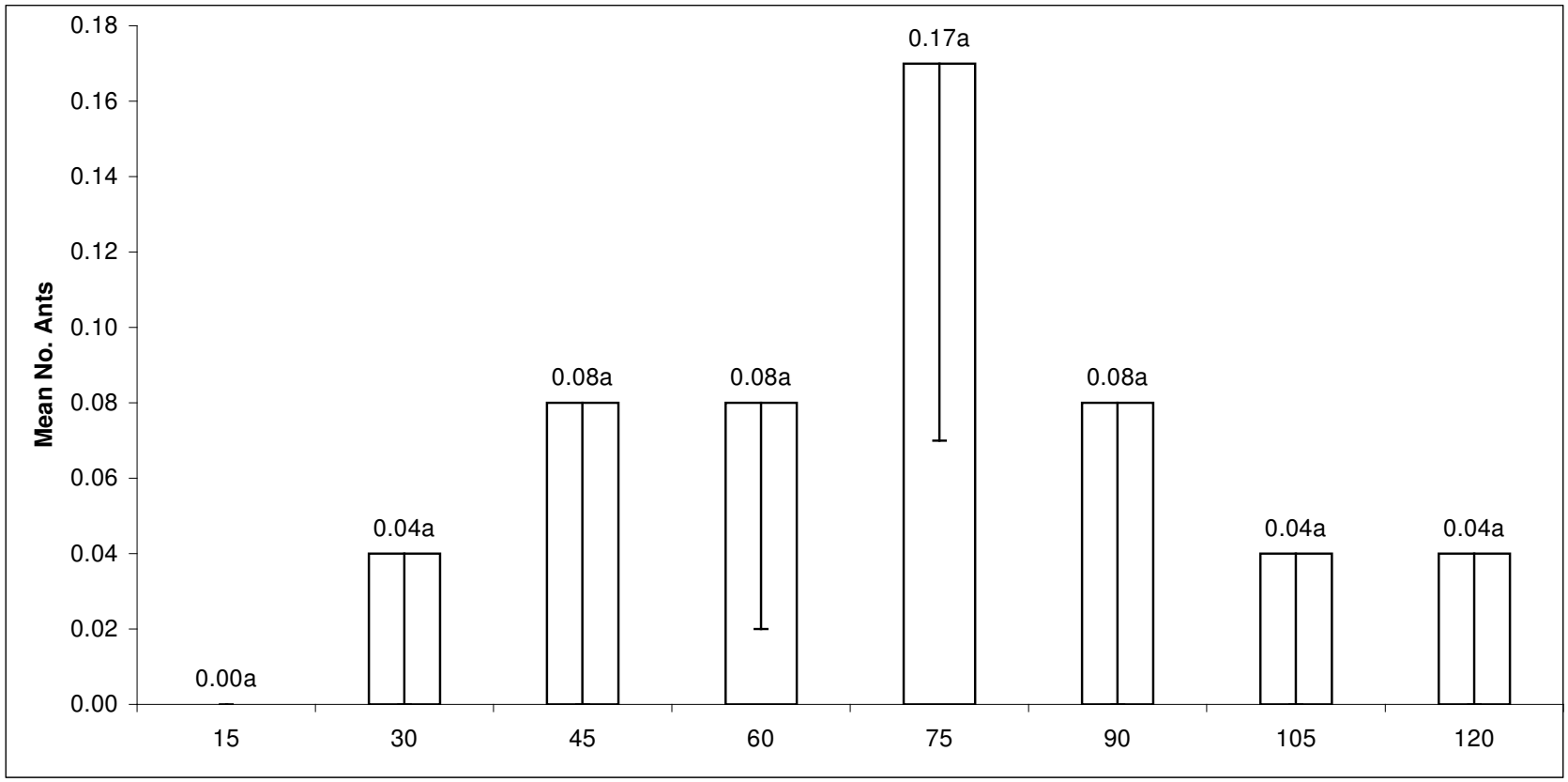

Figure 5-16. Mean number of ants feeding on human plasma among 15 min intervals over the 2 h test period. Means followed by same letter are not significantly different ($P = 0.05$; SAS Institute 2000) and Tukey's HSD test.

CHAPTER 6
SUMMARY AND CONCLUSIONS

The disease vectoring potential of Pharaoh ants, like that of other arthropods more traditionally associated with disease transmission, such as cockroaches and flies, is directly linked to their food preferences and feeding behavior. Therefore, Pharaoh ant consumption of liquid substances that are specifically used in patient care needs to be examined more closely. The goal of this research was to study Pharaoh ant consumption and preferences for patient-care fluids, and to determine what fluid(s) used in patient care attract Pharaoh ants. I accomplished this research by conducting three distinct experiments.

Monomorium pharaonis (L.), the Pharaoh ant, is a pest species which inhabits buildings throughout the world; its populations are difficult to control. It is a highly synanthropic (strongly associated with humans) (Edwards 1991) pest tramp species, and is proclaimed as the "most widely distributed of all ant pests" by Edwards and Short (1990), who summarized its documented presence in the United States, Canada, South America, Egypt, North Africa, Europe, Russia, Australia, and Japan. When in hospitals and other health-care environments, these ants are often found nesting throughout the hospital, in places as diverse as operating rooms, linen closets, baby incubators, and inside unopened intravenous tubing packages. Pharaoh ants have been observed recruiting to human secretions, intact epidermis, and patient-care fluids, such as dextrose and saline solutions. The objective of this study was to determine the Pharaoh ant consumption of patient-care fluids, including human blood, human plasma, 5% dextrose,

0.9% NaCl, and Ensure® dietary supplement, in both no-choice and choice situations, and to determine their recruitment to these fluids in their desiccated state.

Pharaoh ants engage in extensive foraging and are most active at night (Hedges 1997). They probably use "guideline orientation" to follow edges and cracks of structures to find food sources, similar to that observed in *Camponotus pennsylvanicus* and *Tapinoma sessile* (Klotz and Reid 1992). They will also forage via ducts and wall spaces aluminum window and door frames, and they can forage up to distances of 45 m (Vail and Williams 1994).

Pharaoh ants are well-known for their consumption of a wide variety of foods (Edwards and Baker 1981a). Overall, the Pharaoh ant nutritional needs are complex enough to indicate that they do need each of the major nutritional groups (lipids, carbohydrates and proteins), but the Pharaoh ant is an omnivorous carnivore (Ebeling 1978). Some researchers believe that the Pharaoh ant is primarily attracted to sugars (Granovsky and Howell 1983), while others think that they primarily prefer to consume proteins (Edwards and Abraham 1990).

No-choice replications were conducted by placing starved (2 d) Pharaoh ant colonies in an arena with one fluid-loaded microcapillary tube. Test arenas were paired with adjacent control arenas to comprise an experimental unit. Choice replications were performed in the same manner except that 5 fluids were provided simultaneously. Workers foraged from the nesting cells to the microcapillary tubes in order to obtain food and water. Consumption was measured at 24 h.

No-choice studies demonstrated that Pharaoh ants consumed significant amounts of all patient-care and human fluids provided. Consumption of 5% dextrose (37.95 mg)

was significantly higher than that of all other fluids. Consumption of baseline water controls was 2.54 mg.

Choice studies demonstrated that Pharaoh ants consumed significant amounts of all patient-care and human fluids provided. Consumption of 5% dextrose (49.14 mg) was significantly higher than that of all other fluids. Blood was least consumed at 2.05 mg.

Starved (3 d) Pharaoh ants recruited to dried residues of patient-care fluids. Foraging peaked at 45 min after placement, with a mean number of 3.42 ants per substance, and declined to an average of 1.85 ants per substance at 120 min after placement. Foraging to Ensure® dietary supplement (13.89 ants) was significantly highest, followed by recruitment to 5% dextrose (1.49 ants). Few ants (0.03-0.07 ants per substance) recruited to plasma, blood cells or saline.

This study demonstrated that Pharaoh ants occurring in hospitals are capable of consuming patient-care fluids, especially 5% dextrose in the liquid state and Ensure® in the desiccated state. Recruitment and consumption occurred despite the *ad libitum* provision of water in test arenas.

Pharaoh ants live in hospitals, and have been observed feeding upon patients as well as upon patient-care fluids. Their strong synanthropic behavior increases their potential to transmit nosocomial and other infectious diseases to humans in hospital environments. Control is difficult due to the reproductive strategies of this insect (Edwards 1986). The health threat which they pose can be effectively decreased by reducing spillage of patient-care fluids, especially high-sugar fluids such as 5% dextrose and Ensure® dietary supplement.

APPENDIX A
ANT MAINTENANCE

All parent ant colonies (> 200,000 workers) were housed and reared at the Urban Entomology Laboratory, University of Florida, Gainesville, FL. Rearing temperatures ranged from 21.9°C – 29.4°C (71.5°F - 85°F) and relative humidity fluctuated between 20-53%. Attempts were made to control room temperature and humidity by running a space heater 24 hours per day and filling a humidifier once or twice per day as needed to compensate for the room's extreme airflow (rearing room connected to offices with integrated airflow and used outside, cold winter air for air-flow replenishment).

Each colony was housed within a large (40 by 52 by 12 cm deep) plastic tray (Model No. 400-5N, Panel Controls, Greenville, SC). Tray sides were coated with Fluon™ (Asahi Glass Fluoropolymers, Downington, PA) to prevent ant escape. Four to eight cells (depending on the size of the colony) were provided as harborage. Cells consisted of square plastic Petri dishes (100 by 100 by 15 mm) (Williams 1990) with a circular (50 by 2 mm deep) depression in Castone dental plaster (Model No. 99045, Dentsply International, York, PA). Cell lids contained 4 mm holes in each corner to provide ants with multiple ingress and egress points. Yellow or red acetate sheets were taped on top of cell lids, ostensibly to increase desirability of harborage to the ants. Acetate sheets that fell off over time (ants consumed tape and built nests between acetate sheets and lid) were not replaced. In all cases, lids of cells became completely coated with hard, brown, finely-grained ant regurgitation and partially digested food (Peacock and Baxter 1949).

Water and 10% sucrose were provided *ad libitum* in 15 ml vials (Model No. 55-9, Thornton Plastics, Salt Lake City, UT with three large cotton balls inserted into the top to prevent leakage and to provide easy access for the ants. Number of vials varied between colonies based on number of cells within each colony: one vial of water and one vial of sucrose was provided for each nesting cell within parent colony. Water and sucrose vials were replaced one-two times per week (as needed, based on visual determination of fluid quantity remaining, presence of mold or tunneling by ants).

Freshly killed (frozen) American cockroaches, *Periplaneta americana* (Urban Entomology Laboratory, University of Florida, Gainesville, Florida) were provided two-three times per week for protein. Thawed adult and immature *P. americana* were placed in a small soufflé cup (29.5 ml, Model P100, Solo Company, Urbana, IL) with sides coated with Fluon™. All old food was removed, frozen, and disposed of when new food was provided, to prevent mold growth, phorid fly development, and ant-nesting in insect corpses.

Ant colonies were superficially cleaned of dead ant piles and food debris every week. More thorough cleanings of ant regurgitation and partially digested food pellets were performed once per month, and were performed by removing all vials and food from the large plastic tray, stacking cells atop one another at an angle (keeping entrance/exit holes always open) at one side of the tray, and quickly wiping tray bottom with a large, damp cotton ball to pick up regurgitated matter and insect debris. Once clean, a large, dry cotton ball was wiped over the same area to remove any remaining water that might drown the ants. The second side of the large plastic tray was cleaned in

a similar manner. After cleaning, all cells were placed gently back into original position and food, water and sucrose were also returned to colony.

APPENDIX B
SELECTION OF ANTS

Each test unit was populated with 125 outside workers to ensure presence of foragers, 125 inside workers to ensure presence of nurses (Erpenbeck 1976, Mirenda and Vinson 1981, Haack 1987), 3 dealate queens, and 50 mg brood (pupae, larvae and eggs). No attempt was made to include specific proportions of third instars. Following the method of Oi et al., (2000), I randomly selected larvae to comprise ~75% of brood with all larval stages present. Eggs accounted for ~25% of brood.

Foraging ants were selected first by placing a 10 cm by 10 cm piece of white photocopy paper on top of water vials in large (> 200,000 workers) parent colony to collect foraging ants. Ants were then shaken off paper into an empty opaque plastic storage box (18 cm by 32.5 cm by 10.6 cm deep; Model No. 1652, Sterlite Corp., Townsend, MA). Storage box interior sides were coated with Fluon™ (Asahi Glass Fluoropolymers, Downington, PA) to prevent escape. Ants were aspirated out of storage box and transferred to a small soufflé cup (29.5 ml) with Fluon™-coated side. Five different batches (colonies) of 125 ants each were collected (one for each test arena) and placed into individual cups. When each of the five cups contained 125 foragers, all cups containing ants were placed into a storage box and set aside until nurses and brood were collected.

Nurses and dealate queens were selected by emptying individual parent colony rearing cells into an empty storage box with sides Fluon™-coated to prevent escape. Ants not carrying brood (Haack 1987) were aspirated out of this arena. As with foragers,

125 nurses were collected and placed into each of five small soufflé cups. Three dealate queens were then aspirated and placed into each cup along with nurses. These cups were then placed into a storage box and set aside during collection of brood.

Brood of various ages were collected; any ants remaining with brood were manually separated from brood and returned to parent colony. Brood (50 mg, all stages) were weighed on a digital scale and placed into each small cup containing nurses. Nurses and queens within each cup were permitted 1 h to organize brood. After 1 h adjustment time, one entire cup was emptied into each arena by turning the cup upside-down over covered Petri dish (nesting cell) and tapping lightly so that nurses, queens and brood landed on top of harborage cell. Immediately afterwards, foragers were emptied into test arena in same manner. Arena was covered with plastic lid. Placement into arena began starvation period. Ants were starved in test arena 48 h prior to test. Water was available *ad libitum* during the entire starvation period.

APPENDIX C
CONSUMPTION FORMULAS

Fluid loss in test arenas was comprised of consumption and evaporation; therefore, to determine consumed quantities, the amount of fluid lost to evaporation must be subtracted. Many researchers have designed formulas to adjust consumption for fluid loss, and I examined each of the following before selecting and modifying Waldbauer's (1968) formula. Initially, I measured consumption by placing a metric ruler (mm scale) alongside a 30 µl (1 mm ID) microcapillary tube in a method similar to that of (May 1985a, 1985b). This method was quickly abandoned when I determined that gravimetric measurement of fluids was more precise for the small volumes of fluid I was using. Therefore, I considered calculating consumption by gravimetric measurement (Evans 1939, Wei and Johnson 1996). Evans'(1939) formula was based on the assumption that the natural moisture loss rates of leaves kept in containers without insects was similar to that of leaves provided as food to insects, and he controlled consumption for natural moisture loss by taking the difference of uneaten and introduced food weights and multiplying by moisture loss rate:

(% wt loss of aliquot) x (wt food introduced + wt uneaten food) / 2

Any assumptions Wei and Johnson (1996) may have used in the development of their gravimetric method to determine adult Lepidoptera consumption of sucrose solutions via a microcapillary tub were not reported.

Microcapillary tubes provide one of the most controlled methods for liquid food consumption studies. In a tube, water loss occurs only as evaporation from the open ends, in contrast to a leaf, where surface area decreases with consumption so that water loss is affected by changing transpiration and metabolism in addition to evaporation. While the subtraction method is simple and intuitive, it does not take into account the fact that evaporation rates of fluids can change over time as fluid loss occurs (Evans 1939, Candy and Baker 2002). It also does not account for potentially increased surface area of desiccating fluids. Evans (1939) determined a weight loss ratio by multiplying the percent weight loss in control arenas by the mean weight of provided and remaining food in test arenas. As mentioned in Candy and Baker (2002), this adjusts consumption only for evaporation rates of initial weight of food provided in control arenas. Waldbauer (1962, 1968) expanded this formula to include adjustment ratios for evaporation rates of both provided and remaining food. Although he assumed that moisture loss decreased by half, Waldbauer's (1968) formula is a standard method of measuring consumption in nutritional ecology literature (Candy and Baker 2002). Therefore, his formula (shown below) calculates consumption, corrected for fluid loss of variable rates, based upon an assumption of moisture loss rates over time:

$$C = (1 - a/2)(W-[L + bL])$$

where

C = true quantity of fluid consumed after adjusted for evaporation,

W =mass of fluid provided in test arena,

L = mass of uneaten fluid and ants remaining in fluid in test arena,

a = ratio of control fluid weight loss and control fluid initial mass,

b = ratio of control fluid evaporation and final mass.

Waldbauer's (1968) formula was based on several assumptions: (1) moisture loss would decrease at a constant rate over the test period so that final moisture loss rates were half the initial moisture loss rates, (2) percent weight loss was independent of food weight, and (3) feeding does not increase weight loss. (Candy and Baker 2002) derived differential equations from Waldbauer's (1968) work for (1) weight loss due to feeding and moisture loss and (2) weight loss of controls due to moisture loss over several days and reported that his formula resulted in negligible consumption overestimates (<0.2%) when percentage of moisture loss of control foods was < 10%, but that consumption over-estimates were statistically significant when moisture loss of control foods was large. My experiment lasted for only one day, so the differential equation model of Candy and Baker (2002) was not applicable. Waldbauer's (1968) overestimation of consumption was not a limitation for my experiment, since percentage of moisture loss in control arenas was <10%. Thus, Waldbauer's (1968) formula accommodated my data set. Waldbauer (1968) studied caterpillar consumption of fresh leaves. Candy and Baker's (2002) formula was based on the following assumptions: (1) percentage weight loss was independent of food weight, (2) feeding does not increase weight loss, (3) feeding rate per caterpillar was constant, and (4) moisture loss rate was constant proportion to leaf weight with an exponential decrease in weight loss.

Because Evans (1939), Waldbauer (1968) and Candy and Baker (2002) all studied consumption of leaves by caterpillars, their assumption that feeding does not increase natural weight loss by creating a freshly cut surface. Although I used liquids, and was

not concerned about freshly cut surfaces, I was concerned about changing ratios of exposed surface area in regard to liquid volume, since either an increased surface area or a decreased volume would result in increased moisture loss rates over time. Surface area increased due to desiccation in blood cells and Ensure®, and remained constant in all other fluids. Feeding resulted in decreased volume in all fluids. Thus, the moisture loss rates of all fluids changed over time, and Waldbauer's (1968) accommodates my data by permitting adjustments for changing moisture loss rates, even though I used liquids rather than freshly cut leaves.

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BIOGRAPHICAL SKETCH

Roxanne Grace Burrus was born on May 7, 1965, at the Bethesda Naval Hospital in Maryland, to James D. and Dawn E. Burrus, while they were living in nearby Virginia. Her parents met in Washington, D.C., as both were active-duty Navy stationed in that city. Her youth was spent in a variety of countries, including Taiwan, Cyprus, and Spain. She has one younger sister, Karen, who was born in Taiwan in 1967. Roxanne attended Pine Forest High School in Pensacola, Florida, where her father completed his 20 years of service in the US Navy. After high school, Roxanne attended the University of Southern Mississippi, where she obtained a Bachelor of Science in mathematics, with a minor in computer science.

Immediately after graduation from college, she joined the US Air Force and was stationed at Hanscom AFB in Bedford, Massachusetts, where her work at the Air Force Computer Acquisition Center (AFCAC) helped to establish Local Area Networks (LANs) and Wide Area Networks (WANs) in various Department of Defense (DOD) agencies. The LAN and WAN technology was cutting-edge technology at the time; WANs helped pave the way for the current internet that is so familiar now, but was relatively unknown then.

After completing her military service, Roxanne remained in New England, and obtained a position at Edwards High Vacuum, a division of BOC Group, LTD., beginning as an Inside Sales Coordinator, and ending as a Database Project Manager. During her years at Edwards, some friends introduced her to the sport of backpacking in

the White Mountains of New Hampshire. While exploring the vast outdoors with them, she became avidly interested in the creatures and plants she observed, and desired to learn more about the interactions and ecology of these biological organisms.

This desire to learn led her to take an educational path that resulted in leaving Edwards to accept a summer internship position helping a PhD student at the University of Maine, Orono, complete a final field season's documentation of insect diversity in both standing and fallen timber. After a summer counting what seemed like millions of collembola and other minute arthropods in soil samples, Roxanne was especially fascinated with insects, and the events that ultimately led her to the University of Florida seemed to fall into place of their own volition following that summer.

She enjoyed learning biology so much that she attended Middlesex Community College to complete an associate's degree, focusing on life sciences. She then immediately transferred to the University of Massachusetts, Amherst, where she obtained a bachelor's degree in biology. While at UMASS, she became interested in preventive medicine and health, and wanted to pursue both entomology and medical interests.

An internet search of medical schools unexpectedly turned up the US Navy's website describing a field called medical entomology. By becoming a Medical Entomologist in the US Navy, she could be an entomologist and help prevent arthropod-transmission of human diseases. The pivotal decision to become a Medical Entomologist led Roxanne to the University of Florida, where she completed a master's degree in medical and urban entomology in preparation for her career in the Naval Medical Services Corp (MSC).