

The effect of age and diapause on the long-term intake of protein and sugar by two species of blowflies, *Phormia regina* (Meig.) and *Protophormia terraenovae* (R.D.).

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PHORMIA REGINA (MEIG.) AND PROTOPHORMIA
TERRAENOVAE (R.D.).

A Thesis

by

Sharon Lee Greenberg

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August, 1974

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ABSTRACT

The effect of age, sex, diet, and treatment (non-diapausing versus diapausing) and their interactions on the long-term intake of protein and sugar by two species of blowflies, Phormia regina (Meig.) and Protophormia terraenovae (R.D.) were investigated with statistical analysis of the results. Non-diapausing P. regina and P. terraenovae showed similar results. In general, intake was found to be significantly influenced by the age of the fly. Intake was initially low following emergence, increasing rapidly over days 2 to 4 and remaining at a high level (over the first third of the experiment) and then decreasing gradually to the last day of the experiment. Both sexes showed similar trends (patterns) with respect to age, and sugar intake was more affected by the age of the fly than was protein intake. The intake of sugar by males and females of both species was found to be significantly greater than protein intake. However, females consumed more than males of both protein and sugar.

The second part of this study dealt with the question of feeding in diapausing flies. P. regina exhibits an imaginal diapause which may be induced in the laboratory under short photophase and concomitant low temperature regimes. This study represents the first report of a laboratory induced imaginal diapause in the arctic blowfly P. terraenovae. Diapause in this blowfly occurs in response to a short photophase and appears to be temperature independent in contrast to P. regina. Diapause is characterized in both species

by arrested ovarian development in the females and hypertrophied fat body in both sexes. In addition, the development of crop hypertrophy concurrent with the development of the hypertrophied fat body was found to occur in P. terraenovae. This was not found in P. regina.

The results of this study showed that the feeding patterns of the two species differed in several respects when compared to each other and to their non-diapausing counterparts. In general, however, the populations of diapausing P. regina and P. terraenovae were found to feed on sugar and protein and, as was seen similarly in the non-diapausing counterparts of both species, the sugar intake was significantly greater than that of protein. However, whereas the total intake of protein and sugar by diapausing P. regina was found to be significantly greater than that of the non-diapausing P. regina, the opposite was seen for the two experimental populations of P. terraenovae. Non-diapausing P. terraenovae were found to feed significantly more than diapausing P. terraenovae. Furthermore, in both species of flies protein intake by the non-diapausing population as compared to the diapausing population was not significantly different. However, in P. regina, sugar intake was significantly greater in the diapausing flies than in the non-diapausing flies, while for P. terraenovae the reverse situation was seen; sugar intake for the non-diapausing population was greater than for the diapausing population.

A further difference between the two species of diapausing blowflies was seen in the intake of males as compared to females.

Whereas, diapausing female P. regina fed significantly more than diapausing males, no significant difference was seen in the intake of diapausing P. terraenovae males as compared to females.

Furthermore, there was a differential effect of age on the sexes in P. regina, intake of females being more affected by the age of the fly than that of males. However, no significant difference was seen in the effect of age on the intake of diapausing P. terraenovae males as compared to females.

The intake of sugar and protein was significantly influenced by the age of the fly in diapausing P. regina and P. terraenovae; and, furthermore, the intake of both diapausing species of blowflies was found to show greater age effects (to be more influenced by the age of the fly) as compared to their non-diapausing counterparts. In diapausing experiments with P. regina and P. terraenovae, the intake of sugar was found to be more affected by the age of the fly than was the intake of protein; and, in addition was more affected by the age of the fly in the diapausing populations of both species than in their non-diapausing counterparts. The significance of these results are discussed.

GENERAL INTRODUCTION

The feeding behavior and mechanisms regulating food intake in the blowfly Phormia regina (Meig.) have been studied extensively (cf. Dethier, 1969). Regarding dietary self-selection, however, the only long-term qualitative and quantitative studies on the intake of protein and carbohydrate were undertaken without properly controlled environmental conditions or statistical analysis of the results (Dethier, 1961; Gelperin and Dethier, 1967).

Specific hungers and dietary self-selection have been studied in blowflies by Dethier (1961), Strangways-Dixon (1961) and Belzer (1970) who reported a selective increase in protein uptake by the female blowfly prior to vitellogenesis. Dethier (1969) concluded that the specific protein hunger seen in female flies was the result of a protein deficit and that peaks of protein consumption were abolished by any procedure which prevented egg development. During adult diapause in P. regina, vitellogenesis does not take place and ovarian development is arrested. In addition, the fat body of the adult hypertrophies (Stoffolano, et al., 1974). As the effect of diapause on the intake of protein and sugar by the blowfly had not been investigated, it seemed that a study was needed to reevaluate the long-term intake of protein and sugar by blowflies under controlled environmental conditions and with a statistical analysis of the results. This was done using both non-diapausing and diapausing adult P. regina and its northern counterpart, Protophormia terraenovae (R.D.).

This thesis is written in the form of a research paper entitled "The Effect of Age and Diapause on the Long-term Intake of Protein and Sugar by Two Species of Blowflies, Phormia regina (Meig.) and Protophormia terraenovae (T.D.)." In addition, a general literature review and list of references cited are included as well as other pertinent data.

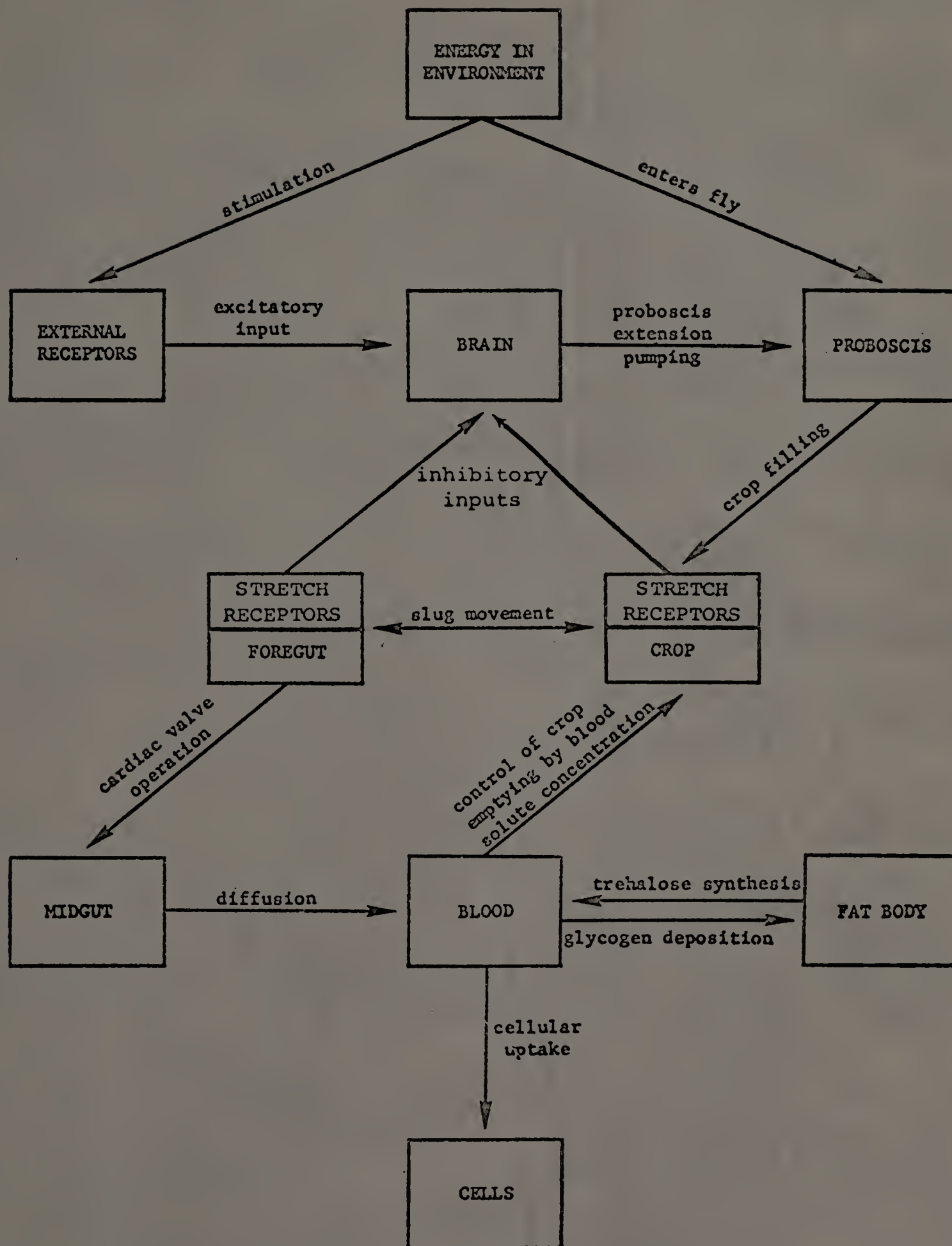
LITERATURE REVIEW

Blowflies are of economic importance in disease transmission and the occurrence of wound myiasis in man and animals (Bishopp, 1915; Hall, 1947; Norris, 1965; Greenberg, 1971, 1973). In addition, some blowflies have been used medicinally in the treatment of wounds because of a healing action associated with the feeding of the larvae (Miller, et al., 1932). In recent years, blowflies have become important model systems for the study of diverse biological phenomena in the fields of physiology, morphology and behavior. The flies represent a natural choice for such studies not only because of their economic importance but because of their value as model systems. Blowflies are comparatively inexpensive and easy to rear, have short generation times and the potential to produce large number of offspring in each generation.

The blowfly, P. regina, has been used as a model system for the study of feeding behavior. Today, the most extensive analysis of feeding behavior and the mechanisms associated with the regulation of food intake, in any animal, has been made utilizing this species of blowfly (cf. Dethier, 1966; Dethier, 1967; Dethier, 1969; Gelperin, 1971).

A. Control of Sugar Intake in the Blowfly

P. regina has been characterized by Dethier as a "feeding machine" requiring only oxygen, water and carbohydrate to survive. Additionally, however, a protein source has been shown to be essential for reproduction. Gelperin (1971) has presented a model, illustrated on the following page, summarizing the proposed mechanism



of control of sugar intake in P. regina. The initiation and termination of feeding, with regard to single meals, are under the control of external chemosensory receptors located on the tarsi, the external surface of the proboscis and internally in the pharynx. When the fly steps in the food, represented in the model as energy in the environment, external chemosensory receptors located on the tarsi send excitatory input to the brain which results in extension of the proboscis and feeding. Ingested food is diverted initially into the crop, a diverticulum of the foregut. Internal sensory receptors located on branches of the abdominal nerve cord function as body wall stretch receptors to monitor crop distension. Negative feedback (seen as inhibitory input in the model) from body wall stretch receptors is received by the central nervous system via the ventral nerve cord and results in an elevated tarsal acceptance threshold and inhibition of feeding.

A second set of internal stretch receptors providing negative feedback to feeding behavior in the fly are located on a branch of the recurrent nerve which connects with the foregut (Gelperin, 1967). These receptors monitor expansion of the foregut such as when food is moved by peristalsis from the crop into the foregut and thence into the midgut. The food solution in the midgut empties by diffusion into the blood. Emptying of the crop is controlled by blood solute concentration (Knight, 1962; Gelperin, 1966). Trehalose is the major blood sugar in P. regina, accounting for a significant fraction of the total blood osmolarity, and is synthesized in the fat body (Clegg and Evans, 1961). As trehalose is removed from the

blood by cellular uptake, the concentration of trehalose and thus the osmolarity of the blood decreases. The contents of the crop are emptied by "slug movement" into the midgut to replace the food lost from the midgut by continual diffusion into the blood.

Friedman (1967) has shown that trehalose synthesis exhibits end product inhibition such that increased concentrations of trehalose in the blood cause a shift in the metabolism of glucose from fat body trehalose to glycogen. During periods of starvation, when food is not available, glycogen is metabolized for the synthesis of trehalose.

B. Specific Hungers Associated with Reproduction.

Important initial questions concerning the regulation of sugar intake have been answered for the blowfly P. regina. Because of this groundwork, further questions about specific hungers, such as those which occur during reproduction, aging and diapause, can be answered using P. regina.

Specific hungers have been known to occur among insects during periods of egg development. While some insects are autogenous, that is they are capable of producing developed eggs on a carbohydrate diet, others require a protein meal for egg maturation to occur. The female blowfly requires a protein meal for vitellogenesis to take place (Mackerras, 1933; Rasso and Fraenkel, 1954; Orr, 1964a, 1964b, Stoffolano, 1974a). However, it is well known that spermatogenesis is independent of feeding in the male blowfly (Cowen, 1932). A recent study by Stoffolano (1974a), revealed that the accessory reproductive glands failed to develop in males fed on

sugar only. The biological function of the accessory reproductive glands in male P. regina may be similar to that for other dipteran species, that is to produce a fluid which induces oviposition in gravid females.

Behavioral studies on the intake of protein and sugar by reproductively active blowflies have revealed that females selectively increase their protein uptake prior to vitellogenesis and that the intake of protein by females is greater than that of males (Dethier, 1961; Belzer, 1970). Strangways-Dixon (1961) reported that during periods of protein intake by female C. erythrocephala, carbohydrate intake was relatively depressed. Dethier (1961) and Belzer (1970) examined the problem of specific hungers during egg development in P. regina and found that an initial peak of protein feeding was normal for males and virgin or sterilized females and that protein consumption remained low after the initial peak. Only in normal females, where egg development occurs, do further peaks of protein consumption occur. Strangways-Dixon (1961), Dethier (1961) and Belzer (1970) found subsequent peaks of protein ingestion followed each oviposition resulting in a cyclically recurring preference for protein. Dethier (1961) noted that for P. regina subsequent peaks of protein ingestion (following the first) were abolished by any procedure which prevented egg development. Thus, although allatectomy, cardiectomy, removal of the medial neurosecretory cells and castration had no effect on the initial protein peak or on carbohydrate intake, in all cases subsequent protein peaks were absent.

Dethier (1969) concluded that the specific hunger for protein

seen especially in reproductively active females was the result of a protein deficit. Removal of the ovaries alters subsequent protein peaks simply because there are no developing eggs to create protein demands. Removal of the endocrine glands, which control ovary development, also affect subsequent protein peaks because the absence of hormones also prevents ovary development. Belzer (1970), in an investigation of the control of protein ingestion in P. regina, also considered protein hunger to be generated by a protein deficit. He showed that procedures which disrupted protein synthesis in the fly affected subsequent peaks of protein consumption. Thomsen (1959) has further shown that the endocrine system may control protein synthesis since, in C. erythrocephala, extirpation of the medial neurosecretory cells results in decreased levels of gut protease activity. These flies, however, were found to feed normally on protein. Dethier (1969) stated that the way in which a protein deficit influences sensory input is at present unknown and suggests that the case for hormonal control may bear further investigation.

C. Effect of Aging on Insect Physiology and Feeding Behavior.

Regarding aging in insects, the nature of processes which limit imaginal lives seem to have the common property of being processes which operate in a cellular system where little or no morphogenetic developments are occurring (Comfort, 1964). Insects present a remarkable phenomena in that the death of the organism coincides with cellular death (Lints and Lints, 1971). As stated by Clark and Rockstein (1964), insects are uniquely suited to studies of the aging process.

The effects or symptoms of aging have been outlined by Clark and Rockstein (1964) and Rockstein and Miquel (1973) to include both structural and functional aspects. Structural changes may occur through mechanical "wear and tear" and involve damage to the exoskeleton and wings. Internal structural alterations may include degenerative changes in the fine structure and number of neurons in the central nervous system (Hodge, 1894; Rockstein, 1950; Herman, et al., 1971; Sohal and Sharma, 1972). Associated with structural changes are a general decline in locomotory capacity, especially flight ability (Williams, et al., 1943; Tribe, 1966), a decline in receptor functioning (Rees, 1970; Stoffolano, 1973) and inefficient metabolism (Tribe, 1967). Physiological changes which accompany aging include reduced reproductive potential (Greenberg, 1955; Woke, et al., 1956; Callahan, 1962), reduced levels of protein synthesis (Levenbook and Krishna, 1971) and a decline in respiratory rates (Bowler and Hollingworth, 1966; Lints and Lints, 1968; Callabrese and Stoffolano, 1974a). Furthermore, the differential effects of aging are a well known phenomena. Males consistently show higher mortality rates as compared to females (Glaser, 1923; Cowan, 1932; Miller, et al., 1932; Rockstein, 1966).

The effect of age on neuronal degeneration has been studied in peripheral sense organs as well as in the central nervous system. Alterations of thresholds and sensitivities for peripheral receptors may be important since these receptors are involved in feeding and, therefore, their functioning is vital to the survival of the fly. As stated by Stoffolano (1973) a degenerated receptor

system in aged flies implies that these flies are less efficient feeders since sensory response from contact chemoreceptors determine whether or not a medium is ingested.

Rees (1970) examined changes occurring in the sensitivity of two receptor cell types located on the labellum of the blowfly, P. terraenovae. He monitored electrical responses of these cells and noted a decline in discharge frequency and an increase in the number of non-functional cells with increasing age.

Rees (1970) also noted in the same report that the mortality plot for these flies showed a similar time course to the plot for receptor failure and suggested that chemoreceptor failure may be an important factor in determining the life span of the fly. He further stated that "...it is difficult to understand how a fly could feed at all if there were no peripheral chemosensory drive to initiate the reflex opening of the labellar lobes or the eversion of the proboscis." He concluded that the changes in sensitivity of the two receptor cell types which appeared to be correlated with age of the adult insect suggest that insect age may need to be considered in a full analysis of feeding behavior in flies.

Stoffolano (1973) showed similar changes in the mean impulse frequency of salt sensilla in the blowfly P. regina with age of the fly. He also showed an increasing number of inoperative sensilla with increasing age in the fly. Furthermore, whereas Rees' (1970) results did not show differences between the sexes, Stoffolano (1973) showed that increasing inoperativity of the salt receptor with increasing age was different for each sex. Males showed a

greater percentage of inoperative sensilla as compared to females of the same age.

There have been no studies designed specifically to investigate the effect of aging on feeding behavior. Several studies do exist, however, in which feeding behavior was studied on a long-term basis and statements regarding the changes which occurred with increasing age have been made.

Greenberg (1959), in a study of housefly nutrition, reported that aging had no effect on the intake of the flies. In addition, he saw no differences in the intake of sucrose by male as compared to female houseflies. Holling (1965), using the mantid H. crassa, noted differential effects of age on the intake of males as compared to females. The consumption by both sexes was initially low and then increased. After the fourth day, however, consumption by males declined steadily while that of females rose to a maximum by the eleventh day. Intake remained relatively constant thereafter for at least another 23 days, when the experiment was terminated. Females ate considerably more than males.

Dethier (1961), in a study of the behavioral aspects of protein ingestion by the blowfly, P. regina, also noted age related changes in food intake and a differential effect of age on the protein intake of the two sexes. Males gradually increased their intake from the time of emergence until the fourth to the eighth day. Intake of protein steadily decreased thereafter until death. Protein intake by females showed a similar pattern except that after each oviposition protein intake increased again. When carbohydrate was the only

material offered to flies, the pattern of daily intake over the life time of the fly was similar for both sexes. Intake of carbohydrate for both sexes was low following emergence, increased to a high level, and then decreased steadily until the death of the fly some 50-60 days later.

Finally, in a study on the long-term regulation of sugar intake by male blowflies, Gelperin and Dethier (1967) showed that intake exhibited a definite aging effect. For the first day or two following emergence, intake was low. Beginning about the second day intake of sucrose increased rapidly and reached a maximum by the second to fourth day. From this day until the death of the fly some 60 days later, daily intake gradually declined. Gelperin and Dethier (1967) stated that the causes underlying the gradual declining in intake with age are not known. "They could be correlated with a gradual decreased activity over the life-span, with a gradual accumulation of stored reserves, or with subtle changes associated with senescence" (Gelperin and Dethier, 1967).

D. The Effect of Diapause on Food Intake

Although several studies have been directed toward an understanding of specific hungers in reproducing insects, little work has been done regarding specific hungers which occur in the diapausing adult. Dethier (1969) concluded that the specific hunger for protein seen especially in reproductively active females was the result of a protein deficit and that procedures which disrupted ovarian development would affect protein hungers. During adult diapause, the reproductive organs do not develop. Thus, the occurrence of this

physiological state would seem to be an excellent opportunity for the further investigation of the question of specific hungers.

Several excellent discussions of diapause have been presented (cf. Andrewartha, 1952; Lees, 1955; deWilde, 1962; Danilevskii, 1965; Beck, 1968; Mansingh, 1971). Adult diapause is characterized as an endocrine deficiency syndrome (deWilde, 1962) manifested by the lack of ovarian development in the female and fat body hypertrophy in both sexes. In addition, Stoffolano (1974a) has shown that in P. regina the reproductive accessory glands fail to develop although spermatogenesis appears to occur normally in diapausing males. The lack of growth and reproductive development associated with diapausing insects represents a changed physiological state with concomitant changes in metabolism. This is manifested by changes in respiratory rates (Lees, 1956). The respiratory rates of diapausing adults as compared to their non-diapausing counterparts have been shown to be much reduced (Slama, 1964; Callabrese and Stoffolano, 1974b).

Accompanying the changes in the physiology of the organism are changes in its behavior. Diapausing adults may refuse to mate (Blondheim and Broza, 1970; Žďárek, 1970), migratory behavior may occur (Nuorteva, 1966; Brown and Chippendale, 1974) as well as changes in the feeding behavior.

The most extensive analysis of diapause feeding has been made amongst the Diptera, specifically the mosquitoes. Experimental work designed to determine if female mosquitoes taking a late fall blood meal prior to diapausing might serve as overwintering reservoirs

for certain viruses, has revealed that blood feeding is generally reduced or absent in the late fall. However, some blood feeding does occur and the term "gonotrophic dissociation" (Swellengrebel, 1929) refers to the condition in which a female mosquito takes a blood meal and doesn't develop ovaries. Spielman and Wong (1973) discuss the adaptive significance of gonotrophic dissociation with regard to overwintering mosquitoes. Consistent with other reports concerning the development of fat body in overwintering insects, Culex pipiens don't appear to become fat until about 2 weeks after entering diapause. Spielman and Wong (1973) state that although the large stores of nutrients held in the fat body of adult mosquitoes are an obvious asset for winter survival, the role of ovarian diapause remains obscure. They suggest that the function of ovarian diapause would be to divert nutrients obtained from a late fall blood meal from the ovary to a storage depot such as the fat body.

Eldridge (1966) noted gonotrophic dissociation to occur in C. pipiens. However, a comparative absence of this phenomena was observed in mosquitoes of the species C. quinquefasciatus. Eldridge (1968) found similar results with these two species of mosquitoes and in addition noted that the amount of fat present in blood fed "dissociated" overwintering mosquitoes was always greater than that in blood fed non-dissociated mosquitoes. These results seem to support the suggestion of Spielman and Wong (1973) that the function of ovarian arrest in overwintering insects is to divert acquired nutrients into the fat body.

Eldridge, et al., (1972) looked at blood feeding and gonotrophic dissociation in two other diapausing members of the genus Culex (C. restuans and C. salinarius). C. salinarius is a more southerly strain while C. restuans is a more northerly one. Expectedly, Eldridge, et al., (1972) found qualitative and quantitative differences in their response to changing photoperiod and temperature. C. restuans showed a drastic reduction in blood feeding in response to fall conditions and exhibited gonotrophic dissociation whereas C. salinarius did not exhibit gonotrophic dissociation under these conditions and showed far less suppression of blood feeding. Eldridge, et al., (1972) attributes these differences in overwintering behavior to differences characteristic of the species and their respective geographic adaptations.

Reports concerning diapause feeding amongst Culex have been presented by Bellamy and Reeves (1963) on C. tarsalis and Sandburg and Larsen (1973) on C. pipiens pipiens. Washino (1970) and Washino, et al., (1971) have examined the question of diapause feeding in mosquitoes of the genus Anopheles. It can be stated in summary that with regard to the question of diapause feeding among overwintering mosquitoes, fall photoperiods inducing overwintering may or may not influence blood feeding. In addition, gonotrophic dissociation is not a general phenomena and it too may or may not occur among diapausing mosquitoes that take a fall blood meal.

A further examination of the literature reveals that many insects do feed when in reproductive diapause although this feeding may be limited to non-protein sources (as in some mosquitoes which engorge on carbohydrates), and to a "pre-diapause" period of feeding.

deWilde (1954) reported a pre-diapause period of intensive food intake and strong fat deposition for the Colorado potato beetle. The ovaries do not develop, however, and at the end of the pre-diapause the beetle ceases feeding and goes into hiding in the soil. Hodek (1971) reported similarly that Pyrrhocoris apterus adults undergo a facultative imaginal diapause in response to short photophases experienced in the fall. The reproductive diapause prevents ovary development in the late summer and early fall during entirely suitable food and climatic conditions before the onset of the adverse conditions. The bugs store reserves in the fat body, cease feeding and go into hiding in litter.

El-Hariri (1965) reported an adult diapause in three species of coccinellidae and stated that their survival during hibernation and in times of scarcity of food must depend largely on the amount of metabolic reserves they can accumulate before hibernating. Mitchell and Taft (1966) reported similarly that the accumulation of large fat deposits by diapausing boll weevils may be coincidental to the occurrence of diapausing in this insect and occurs primarily because the weevils continue to feed at a time when their rate of metabolism and food requirements are considerably reduced.

A recent discussion by Mansingh (1971) on the physiological classification of diapause appears to be of great importance to the question of diapause feeding. Mansingh subdivided diapause into three physiological states; a pre-diapause period (characterized as a preparatory period), a diapause period and a post-diapause period. The preparatory phase is essential for the survival of the insect during

the period of diapause which follows. During the first stage, which lasts 2 to 3 weeks, the insect is involved in accumulating large stores of nutrient reserves. This phase leads gradually to the diapause condition where endergonic biosynthetic and exergonic energy trapping activities such as respiration are at a minimum. The examples of diapause feeding reported by the various authors in the preceeding paragraphs seem to support the model outlined by Mansingh (1971).

Stoffolano (1974b) states that "...it seems obvious that such a period of feeding when large accumulations of food are set aside for the winter should exist", but questions what mechanisms might underlie such behavior. Stoffolano (1968) suggested a hypothesis for the control of diapause feeding by the face fly Musca autumnalis. M. autumnalis exhibits a facultative imaginal diapause characterized by fat body hypertrophy in both sexes and cessation of ovarian development in the females. Stoffolano and Matthysse (1967) have reported that at short photophases and low temperature, diapausing adult face flies failed to feed on cow blood. However, a pre-diapause preparatory period appears to exist in this fly. Flies entering diapause in the fall, instead of seeking cattle, feed mainly on plant nectar and a period of carbohydrate feeding occurs before a full complement of fat results. Stoffolano (1967) reported that fat body hypertrophy did not develop until 14 days after emergence in diapausing adult face flies. In addition, Stoffolano (1968) reported that 96% of the diapausing adult face flies showed hypertrophied crops. It is suggested by Stoffolano (1968) that the same mechanism shown to be operating to limit long-term intake of sugar in P. regina may be

involved to reduce feeding in diapausing face flies with fat hypertrophy. Fat and full crops in diapausing flies act similarly to a full crop or the developed eggs in a gravid non-diapausing female to activate abdominal stretch receptors resulting in elevation of the tarsal acceptance threshold and cessation of feeding.

INTRODUCTION

Insects are good systems to study feeding behavior. In some insects, in particular the black blowfly, P. regina, it has been shown that they regulate food intake. This species has been the subject of feeding studies in which both the maintenance of metabolic homeostasis and the underlying regulatory mechanisms have been investigated. Furthermore, although the principle food of the blowfly is carbohydrate (Dethier, 1969), Strangways-Dixon (1961), Dethier (1961) and Belzer (1970) have demonstrated the existence of a specific protein hunger in flies. Female blowflies have been shown to selectively increase their consumption of proteins prior to periods of egg maturation resulting in the appearance of cycles of protein intake concomitant with reproductive cycles. In addition, although spermatogenesis occurs independent of feeding in the blowfly (Cowan, 1932; Mackerras, 1933; Stoffolano, 1974a), Stoffolano (1974a) has shown that male P. regina require a protein meal for the maturation of the accessory reproductive glands. Belzer (1970) noted that male blowflies exhibited an initial peak of protein consumption but did not show subsequent peaks of protein intake, as was seen in the female.

The diet of the fly appears to be closely correlated with its physiological state. The fly has been shown to be capable of taste discrimination and of selectively increasing needed components of the diet in response to unique metabolic requirements. The design of this study was to observe the effects of two experimentally manipulated physiological states, age and diapause on the long-term intake of the fly.

Studies on aging in insects (Clark and Rockstein, 1964; Rockstein and Miquel, 1973) indicate that there are numerous degenerative structural and functional changes occurring in aging adult insects such as mechanical damage to the cuticle and wings, reduced fecundity, reduced levels of protein synthesis and respiration rates, and degeneration of the central nervous system. Changes occurring in the structure and function of the nervous system, specifically the peripheral chemoreceptor sense organs are of particular interest since these receptors are intimately involved in the feeding process. A receptor system which showed age-related degenerative changes would imply that aged flies are less efficient feeders. Studies by Rees (1970) and Stoffolano (1973) have indicated that aged flies show increasing numbers of non-functional labellar chemoreceptors and that among those that remain functional, a decrease in the mean impulse frequency occurs.

Stoffolano (1973) reported the occurrence of a facultative imaginal diapause in P. regina that was characterized by the lack of ovarian development in the female and by hypertrophy of the fat body in both sexes. In addition, the accessory reproductive glands are undeveloped in the diapausing fly. The occurrence of an imaginal diapause has also been reported for P. terraenovae (Roubaud, 1927; Cousin, 1932; Danilevskii, 1965). Behavioral alterations such as migratory behavior, reduced mating activity and changes in feeding behavior may also occur during diapause. Lees (1955) has stated that prior to entry into diapause many insects feed ravenously to build up large reserves of fat and glycogen in the fat body and

other storage tissues. As stated by Stoffolano (1974b), however, no work has been done on the selective intake of protein and sugar by diapausing flies as compared to their non-diapausing counterparts.

This paper presents the results of an investigation aimed at elucidating the effect of age and diapause on the long-term intake of protein and sugar by male and female P. regina and P. terraenovae maintained under laboratory controlled environmental conditions. Additionally, statistical methods were used in an analysis of the results.

MATERIALS AND METHODS

A. Rearing of Stock Colonies

Adult P. regina and P. terraenovae were collected in 1972; P. regina in Ithaca, New York, and P. terraenovae in College, Alaska. Rearing procedures for both species were identical.

All life stages were reared under 24 h illumination at 24 °C and 50-60% relative humidity (R.H.). Adults had free access to a diet of granulated sucrose and water. Pork liver was offered as a protein source and oviposition medium. Eggs were collected and transferred to 250 ml beakers containing 150 ml of an artificial diet (Stoffolano, 1973). When larvae were mature, sawdust was added to the beakers to facilitate pupation. These beakers were placed in the stock cage where the adults emerged from the sawdust.

B. Rearing of Experimental Colonies

Eggs were collected from the stock colony and reared on the artificial diet. Non-diapausing adult P. regina were obtained from larvae reared at 24 °C, 24 h light and 50-60% R.H. Pupae were transferred to an environmental chamber where subsequent adults were reared.

Non-diapausing P. regina were reared under June conditions of daylength and temperature for the Ithaca, New York area in an attempt to simulate those conditions experienced by these insects in nature. Adults received a 16 h photophase with a temperature maximum of 24 °C during the light phase and a minimum of 13 °C during the dark phase (8 h). The average temperature for the dark and

light phase combined was 17 °C.

Diapausing adults were obtained from flies reared under conditions described by Stoffolano (1973). The larvae were reared in an incubator in total darkness and at a temperature of 24 °C. The pupae formed were then transferred to an environmental chamber where they and subsequent adults received a 9 h photophase at a temperature of 18 °C.

The experiments with P. regina on the intake of protein and sugar by non-diapausing and diapausing flies were repeated with some modifications using the arctic blowfly, P. terraenovae. P. regina requires that a concomitant low temperature and short photophase be experienced by the adult for induction of the diapause condition whereas in P. terraenovae diapause appears to be temperature independent (personal observations) and diapausing flies can be reared at the same temperature as the non-diapausing population thus eliminating a possible differential temperature effect which might confound the results. Therefore, P. terraenovae was used to repeat those studies made with P. regina on the intake of protein and sugar by diapausing and non-diapausing flies.

Non-diapausing adult P. terraenovae were obtained from larvae reared similarly to those of non-diapausing P. regina. Pupae and subsequent adults were transferred to an environmental chamber where they received 24 h illumination and were left at 24 °C. Diapausing flies were obtained from larvae reared in a manner similar to those of diapausing P. regina. Pupae were transferred to an environmental chamber where adults were kept at a temperature of 24 °C with a 9 h photophase.

Large numbers of larvae were reared in order to obtain enough flies of approximately the same age. Only those flies emerging during one twelve hour period were used, all others were discarded. The maximum age difference between any two flies was thus twelve hours.

Adult flies were maintained in approximately equal numbers in 25 X 50 cm wire mesh cages with front sleeving. Paper toweling, which was changed weekly, was placed on the bottom of the cages to absorb liquid wastes. The cages contained standard feeding solutions offered in separate glass vials stoppered with absorbent rolled cotton wicks. Feeding solutions consisted of 10% (w/v) yeast extract (Nutritional Biochemicals Co.) and .1 M sucrose prepared in distilled water. These solutions, offered as standard diet to the experimental colonies were identical to the solutions used in the two-choice testing experiments. The feeding solutions were prepared fresh daily. Water was also available to the experimental colonies and was changed weekly. The artificial diet (Stoffolano, 1973) on which the larvae were reared was used as an oviposition medium in a manner similar to Orr (1964a). This eliminated an additional source of protein in the experimental colonies since flies were observed to oviposit, but not to feed on the medium. Flies were also observed to oviposit readily on the rolled cotton wicks saturated with feeding solutions. The gelled medium which was offered in waxed paper dishes and was changed every other day was prepared ahead of time and refrigerated until needed.

Cages were housed in an environmental chamber equipped with four 20 watt fluorescent lights. Flies received a photophase and

temperature regime as described earlier. The light intensity was 3767.49 lux at the front of the chamber and 2475.78 lux at the back. Humidity was maintained in the environmental chamber at 50-60% R.H.

C. Measurement of Intake

Food intake was measured using a two-choice apparatus, consisting of individual feeding units housed in a clear plexiglass container, adopted and modified from Belzer (1970).

Individual feeding units (Fig. 1) consisted of two precision bore glass capillary tubes (I.D. 0.889 cm, 30.5 cm long) inserted through holes drilled in a rubber stopper which in turn fitted into a cellulose nitrate centrifuge tube, 3 cm in diameter and cut to 7.5 cm in length with nylon mesh glued to the cut end. The capillary tubes, calibrated externally in mm, were obtained from Ace Glass Co., Vineland, New Jersey. The use of these tubes, rather than those of Belzer (1970), provided for more accurate measurement of intake. A further benefit of the calibrated capillary tubes was that it was no longer necessary to open the clear plexiglass container in which the tubes were housed to take readings. Readings could be taken through the plexiglass container, thus humidity inside the container was subject to fewer fluctuations.

The clear plexiglass container (Fig. 2) measured 25 X 36 X 38 cm. The individual feeding units housed in the container were secured with rubber tubing stretched on removable plexiglass platforms. The platforms rested on adjustable wooden racks which could be tilted to produce an incline such that the fluid in the capillary tubes would flow downward as the fly fed. Relative humidity in the

plexiglass container was maintained at 60-70% R.H. with a saturated NaCl solution prepared according to Winston and Bates (1960) and placed in a pan on the bottom of the container. The entire apparatus was housed in an environmental chamber equipped with four 20 watt fluorescent lights. Humidity in the chamber was maintained at 50-60% R.H.

The standard feeding solutions were introduced into the glass capillary tubes using a hypodermic syringe and were then allowed to equilibrate for approximately 2 h during which time evaporation amongst the capillary tubes was found to be the greatest. One tube of each feeding unit contained the 10% yeast extract solution or protein solution; the other contained the .1 M sucrose or sugar solution. A "zero" reading was taken at the end of this period and the cellulose nitrate centrifuge tubes containing the flies were fitted into place. Final readings were taken the next day (22 h later), all readings were taken at the same time each day. Capillary tubes were removed and replaced with clean ones at the end of each feeding trial. Used tubes were washed in detergent solution, rinsed in tap water and dried in an oven at 75 °C.

Seven flies of each sex were used each day in calculating the daily mean intake. Flies were taken from the experimental colonies and briefly anesthetized with CO₂. The sexes were separated, and transferred to the cellulose nitrate centrifuge tubes, one fly to a tube. An equal number, seven, of evaporation controls (consisting of the individual feeding units without flies) were run concurrently.

Evaporation amongst the capillary tubes was found to be less in those tubes closest to the salt bath and greater among those farthest away. Controls and flies were therefore distributed as to their position on the plexiglass platforms using a table of random numbers to minimize variations in the results due to differential evaporation. Intake was measured from day 1 to day 40 for P. regina and from day 1 to day 25 for P. terraenovae. Flies were tested at the same temperature and photophase regime under which they were reared. In addition, in order to remove any effects due to the temperature on feeding, diapausing P. regina were tested at the same temperature and photophase regime experienced by the non-diapausing population.

D. Dissections

Females were dissected in an insect saline solution (Normann and Duve, 1969) at the termination of each day's feeding trial and ovarian measurements taken to determine their relative stage of development. Diapausing flies were also dissected and examined for fat body hypertrophy and ovarian development.

E. Statistical Analysis

An analysis of variance of the data was used to determine the effect of sex, age, diet and treatment (diapausing or non-diapausing) and their interactions on the intake of the fly. This included computer calculation of means, standard error of the mean, and F-values. An analysis of variance was also made of evaporation data and these results used in calculating adjusted daily mean intake values. Each point on the graphs represents the daily

mean intake for 7 flies minus the mean evaporation for that day,
for that diet and for that treatment.

Fig. 1. The individual feeding unit consisting of (a) two precision bore glass capillary tubes inserted through holes in (b) a rubber stopper which in turn fit into (c) a cellulose nitrate centrifuge tube with (d) nylon mesh glued to the cut end. A fly is shown on one of the capillary tubes containing the protein solution (shown in black) while the other tube contains the sugar solution (shown clear).

Fig. 2. The clear plexiglass container housing the individual feeding units. (a) Removable plexiglass platforms rest on (b) adjustable wooden racks. Individual feeding units are secured with (c) rubber tubing. An enamel pan (d) containing the saturated NaCl solution is placed on the bottom of the container. Placement of the individual feeding units within the plexiglass container is indicated; normally the container would hold as many as 21 individual units, seven on each platform.

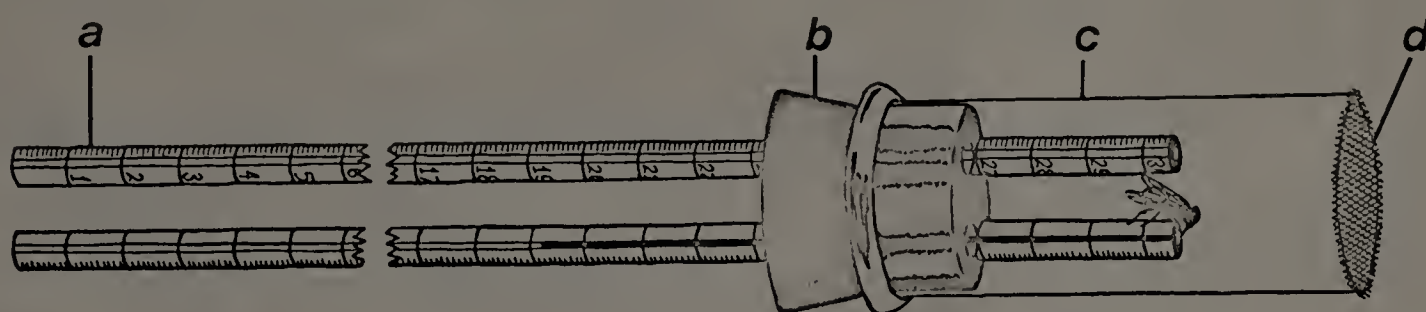


Fig. 1

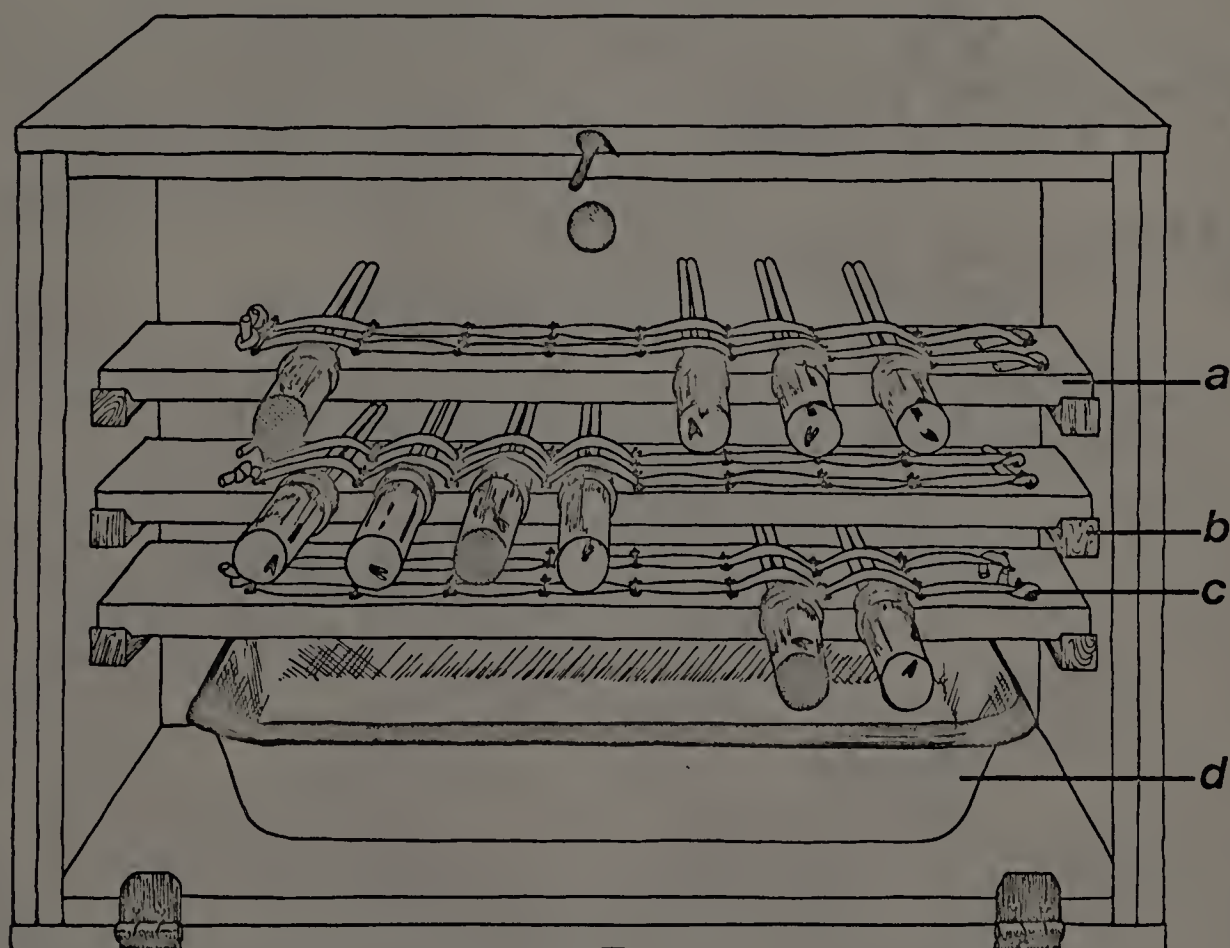


Fig. 2

RESULTS

A. Experiment 1: Non-diapausing P. regina

The following experiment was undertaken to determine the intake of protein and sugar by non-diapausing male and female P. regina from day 1 to 40 days after emergence. The results of this experiment are shown graphically in Fig. 3, while the analysis of variance of these data is presented in Table 1.

Analysis of variance showed age to have a significant effect ($P < .01$) on the total daily mean intake of protein and sugar by the population of flies. Total daily mean intake for the population was low during the first three days, increasing on day 4 through 16 when intake began to gradually decline until day 40. Females were found to have a significantly greater ($P < .01$) total daily mean intake of protein and sugar than males. Total daily mean intake for females was 19.68 μ l as compared to 16.29 μ l for males.

The total daily mean intake of sugar by the population of flies was significantly greater ($P < .01$) than that of protein. Total daily mean intake of protein by the population of non-diapausing flies was 6.66 μ l as compared to 29.32 μ l for sugar. There was no significant Sex x Diet interaction. Total daily mean intake of protein by females was 8.98 μ l as compared to 4.35 μ l for males while total daily mean intake of sugar by females was 30.40 μ l as compared to 28.24 μ l for males. No significant Sex x Age or Sex x Age x Diet interaction was seen. There was, however, a significant Age x Diet interaction ($P < .01$).

Intake of sugar was more affected by the age of the fly than was protein intake.

B. Experiment 2: Diapausing P. regina

The following experiment was undertaken to determine the intake of protein and sugar by diapausing male and female P. regina from day 1 to 40 days after emergence. Dissection of the flies after testing revealed that 85% of the population were in diapause. Diapausing flies were identified on the basis of lack of ovarian development in females or the hypertrophy of the fat body in both sexes although the latter did not become recognizable until day 15 following emergence. The results of experiment 2 are illustrated graphically in Fig. 4.

Analysis of variance of data obtained from experiment 2 (Table 2) showed age to have a significant effect ($P < .01$) on the total daily mean intake of protein and sugar by the population of diapausing flies. Intake was initially low following emergence and then increased rapidly on days 2 to 3. A high level of intake was maintained until days 27 and 28 when intake began to decline until day 40, the last day of the experiment. Females were found to have a significantly greater ($P < .01$) total daily mean intake than males. Total daily mean intake for females was 30.02 μ l as compared to 24.27 μ l for males.

Total daily mean intake of sugar by the population of diapausing flies was found to be significantly greater ($P < .01$) than that of protein. Total daily mean intake for protein was 5.33 μ l as compared to 49.16 μ l for sugar. There was no significant Sex x Diet interaction. Total daily mean intake of protein by females was 8.07 μ l

as compared to 2.59 μ l for males while total daily mean intake of sugar by females was 51.97 μ l as compared to 46.34 μ l for males. A significant Sex x Age ($P < .01$) interaction was shown, the effect of age on the intake of the sexes was not similar. In addition a significant Age x Diet ($P < .05$) and Sex x Age x Diet ($P < .05$) interaction was shown.

An analysis of variance was conducted on data from experiment 1 and experiment 2 in order to make comparisons between the intake of non-diapausing versus diapausing P. regina. This analysis (Table 3) showed that the diapausing flies fed significantly more ($P < .01$) than the non-diapausing flies. Total daily mean intake by the diapausing population was 27.18 μ l as compared to 17.99 μ l for the non-diapausing population. While the protein intake by the two populations of flies was not significantly different, diapausing flies were found to feed significantly more ($P < .01$) on sugar than the non-diapausing flies. Total daily mean intake of protein by the diapausing flies was 5.33 μ l as compared to 6.66 μ l for the non-diapausing population while total daily mean intake of sugar by the diapausing flies was 49.16 μ l as compared to 29.32 μ l for the non-diapausing flies.

An analysis of variance comparing the effects of age on the total daily mean intake of protein and sugar by the non-diapausing and diapausing flies showed a significant difference ($P < .01$) between the two experimental groups of insects, the effect of age being greater in the diapausing group.

Fig. 3. Total daily mean intake (μ l) of protein and sugar by non-diapausing male and female P. regina from day 1 to 40 days after emergence. Each point on the graphs represents the daily mean intake for 7 flies minus the mean evaporation for that day, and for that diet. Arrows indicate days on which eggs were laid.

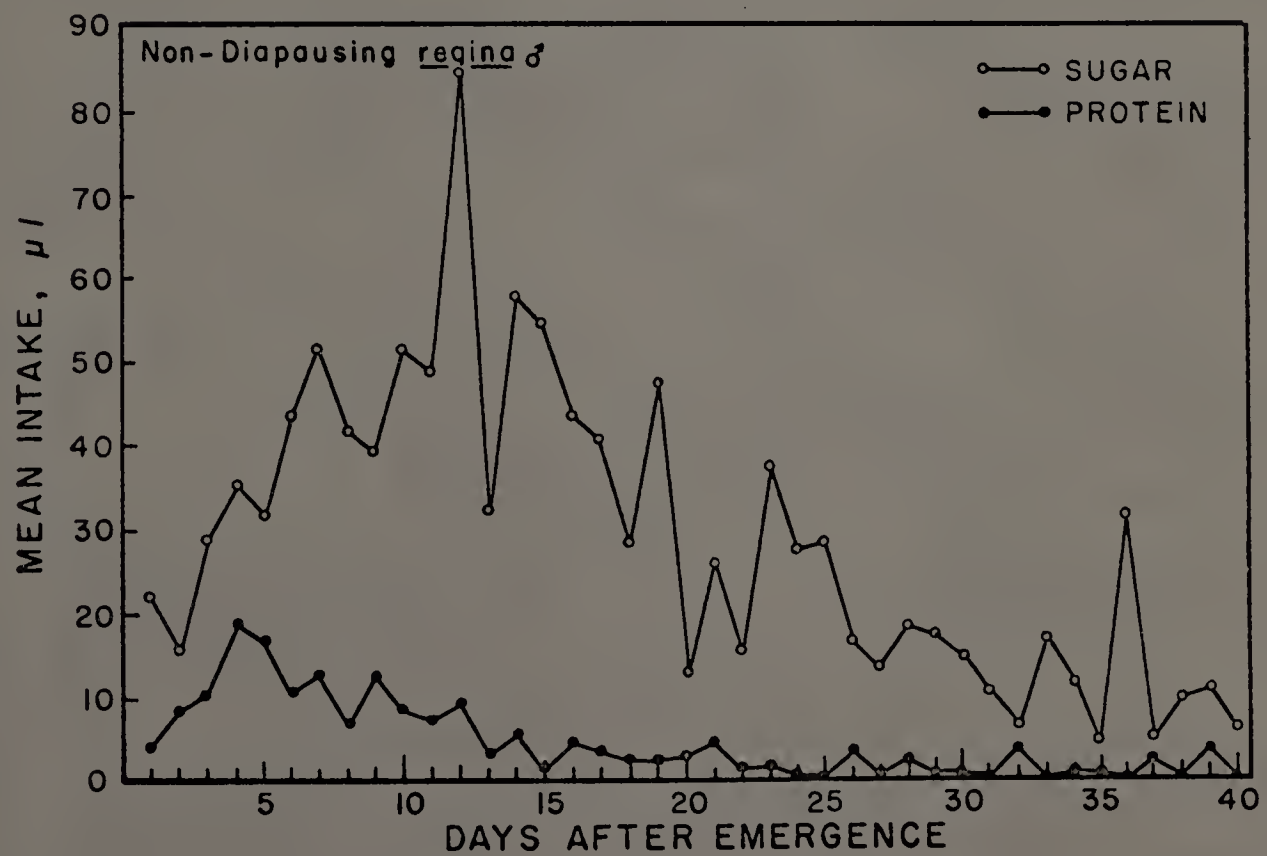
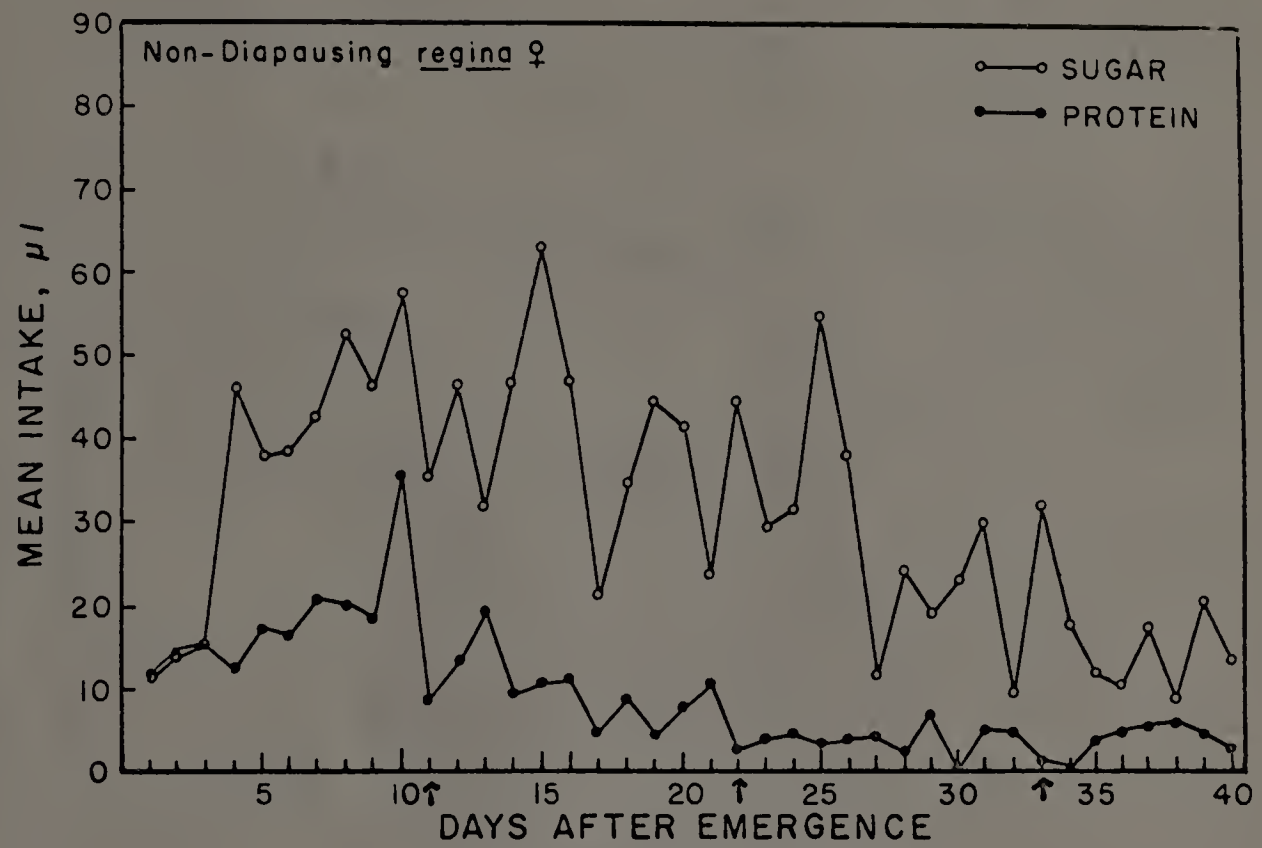


Fig. 3

Fig. 4. Total daily mean intake (μ l) of protein and sugar by diapausing male and female P. regina from day 1 to 40 days after emergence. Each point on the graphs represents the daily mean intake for 7 flies minus the mean evaporation for that day and for that diet.

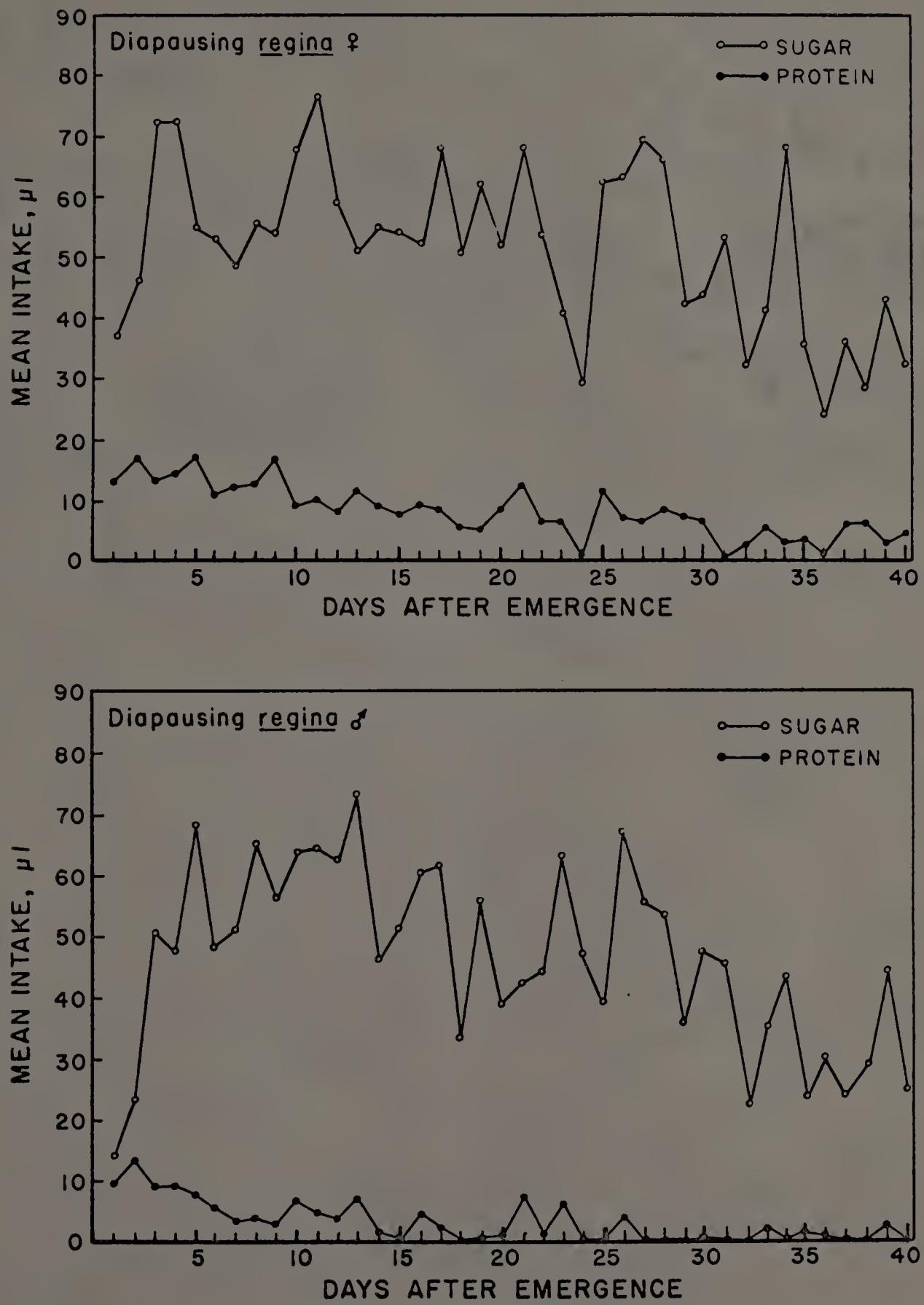


Fig. 4

No significant Sex x Treatment interaction was found; females in both experimental situations fed more than males. There was, however, a significant Age x Diet difference ($P < .01$) between the non-diapausing and diapausing flies. Diet was more influenced by age in the diapausing flies than in the non-diapausing group. No significant difference was found in the Sex x Age or Sex x Diet interactions between the two groups.

C. Experiment 3: Non-diapausing P. terraenovae

Experiments 1 and 2 on the long-term intake of protein and sugar by non-diapausing and diapausing P. regina were repeated with modifications using the arctic blowfly, P. terraenovae. The results of experiment 3 on the intake of protein and sugar by non-diapausing P. terraenovae from day 1 to 25 days after emergence are shown graphically in Fig. 5.

Analysis of variance of data obtained from experiment 3 (Table 4) showed age to have a significant effect ($P < .01$) on the total daily mean intake of protein and sugar by the population of non-diapausing flies. Intake was initially low during the first 1 to 2 days after emergence. This increased on days 2 to 3 and remained at a high level to days 14 to 15 when intake began to decline steadily to day 25, the final day of the experiment. Females were found to have a significantly greater ($P < .05$) total daily mean intake than males. The total daily mean intake for females was 27.47 μ l as compared to 24.45 μ l for males.

Total daily mean intake of sugar by the population of non-diapausing P. terraenovae was found to be significantly greater

($P < .01$) than that of protein. This was similar to what was found for non-diapausing P. regina. The total daily mean intake of sugar by the population of non-diapausing P. terraenovae was 46.26 μ l as compared to 5.65 μ l for protein. There was no significant Sex x Diet interaction. Total daily mean intake of protein by females was 7.42 μ l as compared to 3.65 μ l for males while total daily mean intake of sugar by females was 47.51 μ l as compared to 45.23 μ l for males. A significant Age x Diet interaction ($P < .01$) was seen. The intake of sugar was more affected by the age of the fly than was protein intake. However, there was no significant Sex x Age or Sex x Age x Diet interaction. These results were similar to those for non-diapausing P. regina.

D. Experiment 4: Diapausing P. terraenovae

The following experiment was undertaken to determine the intake of protein and sugar by diapausing male and female P. terraenovae from day 1 to 25 days after emergence. Diapausing flies were characterized by the absence of ovarian development in the females and by fat body hypertrophy in both sexes. The latter did not become apparent until day 13 or 14 and, in addition, the crop was observed to be filled with a clear viscous fluid beginning on approximately day 12 to 14. This phenomena was not seen in the diapausing P. regina population.

The results of experiment 4 on the intake of protein and sugar by diapausing P. terraenovae are illustrated graphically in Fig. 6. Table 5 shows the results of analysis of variance of these data. Total daily mean intake of protein and sugar by the population of

diapausing flies was found to be significantly ($P < .01$) influenced by the age of the fly. Intake was low on day 1, the first day after emergence and then increased rapidly on the second day to a high level which was maintained until day 12 when intake began to decrease rapidly until the last day of the experiment on day 25. No significant difference was found in the total daily mean intake of protein and sugar by diapausing females as compared to diapausing males. The total daily mean intake for females was 21.50 μ l as compared to 21.80 μ l for males. These results are in contrast to those of diapausing P. regina in which a significant difference was seen in the total daily mean intake for females as compared to males.

Total daily mean intake of sugar by the population of diapausing flies was significantly greater ($P < .01$) than the protein intake. This was similar to what was seen in experiments 1, 2, and 3. Total daily mean intake of sugar by the population of diapausing P. terraenovae was 38.11 μ l as compared to 5.20 μ l for protein. There was a significant Sex x Diet interaction ($P < .05$). Total daily mean intake of protein for females was 6.04 μ l as compared to 4.36 μ l for males while total daily mean intake of sugar for females was 36.96 μ l as compared to 39.26 μ l for males. A significant ($P < .01$) Age x Diet interaction was shown, total daily mean intake of sugar was more affected by age than protein which was less variable. There was no Sex x Age interaction, both sexes showing similar trends in intake with respect to age. There was no significant Sex x Age x Diet interaction.

Analysis of variance of data from experiment 3 with non-diapausing

P. terraenovae and experiment 4 with the diapausing population, (Table 6) carried out to make comparisons between the two, showed that the total daily mean intake by the population of non-diapausing flies was significantly greater ($P < .01$) than that of the diapausing population. These results are in variance with those of P. regina where the diapausing population was found to feed significantly more than the non-diapausing population. Total daily mean intake of protein and sugar by the non-diapausing flies was 25.96 μ l as compared to 21.65 μ l for the diapausing population. Total daily mean intake of protein by the non-diapausing population was not significantly greater than that of the diapausing population. However, the total daily mean intake of sugar by the non-diapausing group was significantly greater ($P < .01$) than that of the diapausing group. Total daily mean intake of protein for the non-diapausing population of P. terraenovae was 5.65 μ l as compared to 5.20 μ l for the diapausing population while total daily mean intake of sugar for the non-diapausing population of P. terraenovae was 46.26 μ l as compared to 38.11 μ l for the diapausing population.

Analysis of variance comparing the effects of age on the total daily mean intake of protein and sugar by the non-diapausing versus diapausing flies showed that there was a significant difference ($P < .01$) between the two experimental treatments, the effect of age being greater in the diapausing group. In addition, there was a significant ($P < .01$) Treatment x Sex x Age interaction.

The Sex x Treatment interaction was significant ($P < .05$).

The total daily mean intake of protein and sugar by diapausing males

as compared to diapausing females was not significantly different, 21.80 μ l for males and 21.50 μ l for females, while the total daily mean intake of protein and sugar by non-diapausing males as compared to non-diapausing females was less, 24.45 μ l for males and 27.47 μ l for females. There was no significant Treatment x Sex x Diet, or Treatment x Sex x Age x Diet interaction. The Treatment x Age x Diet interaction was significant ($P < .01$). Sugar intake was more affected by the age of the fly in the diapausing population than in the non-diapausing population.

Fig. 5. Total daily mean intake (μ l) of protein and sugar by non-diapausing male and female P. terraenovae from day 1 to 25 days after emergence. Each point on the graphs represents the daily mean intake for 7 flies minus the mean evaporation for that day and for that diet. Arrows indicate days on which eggs were laid.

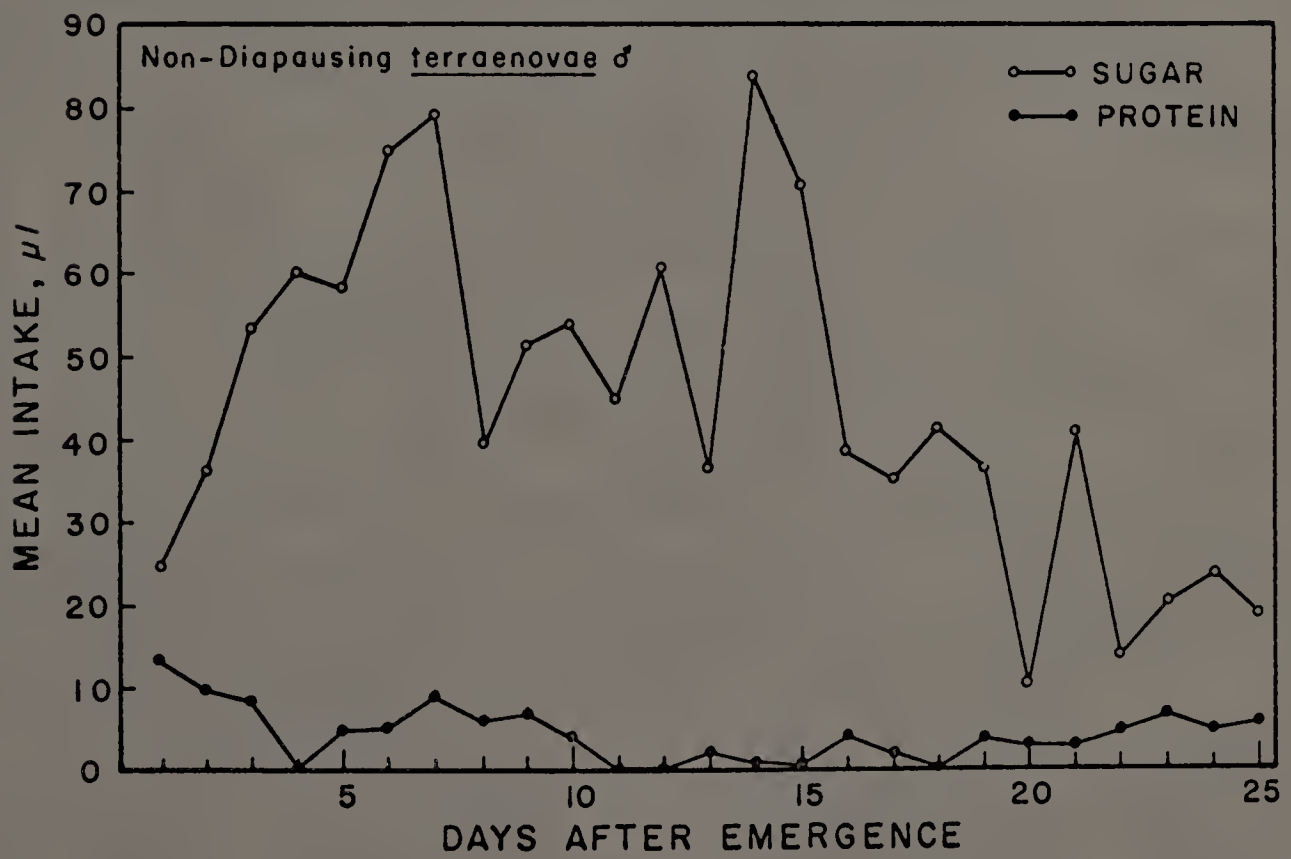
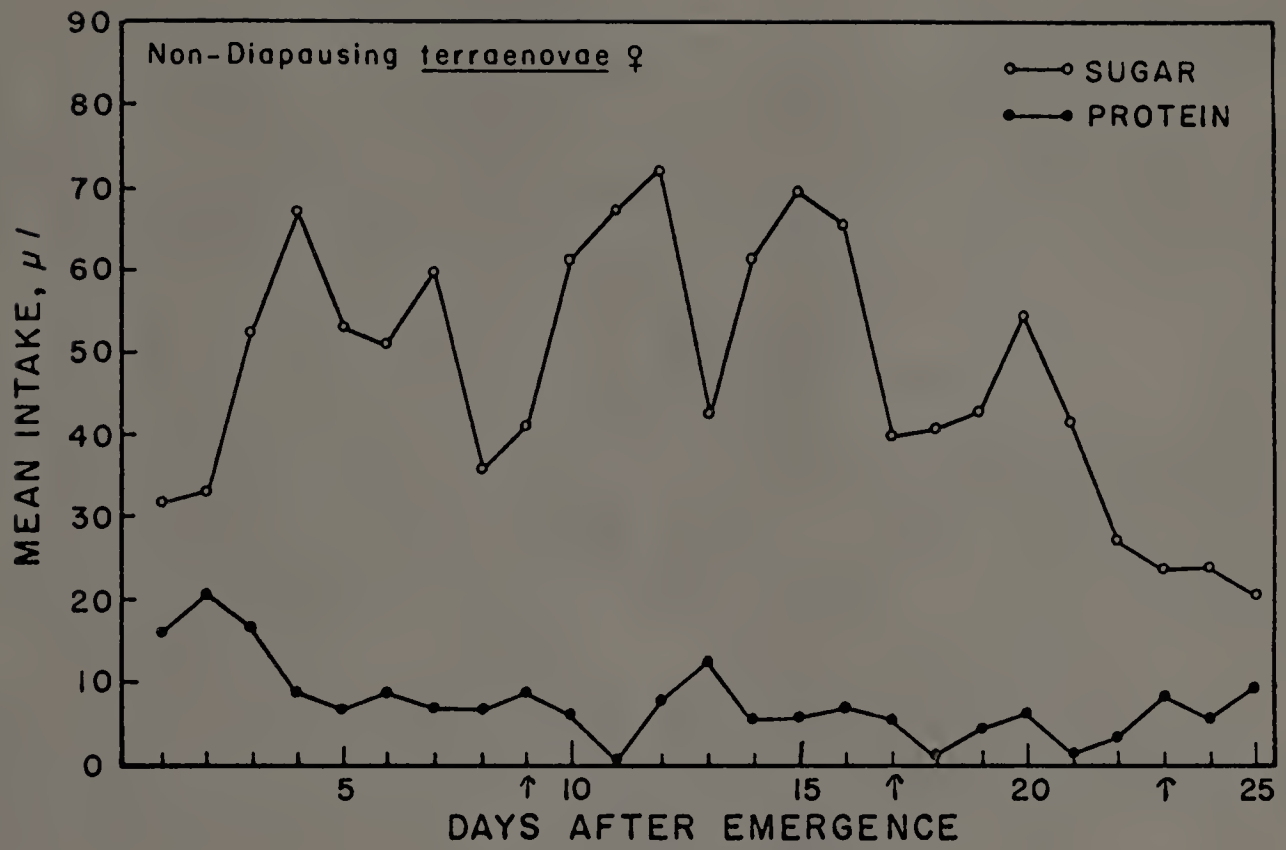


Fig. 5

Fig. 6. Total daily mean intake (μ l) of protein and sugar by diapausing male and female P. terraenovae from day 1 to 25 days after emergence. Each point on the graphs represents the daily mean intake for 7 flies minus the mean evaporation for that day and for that diet.

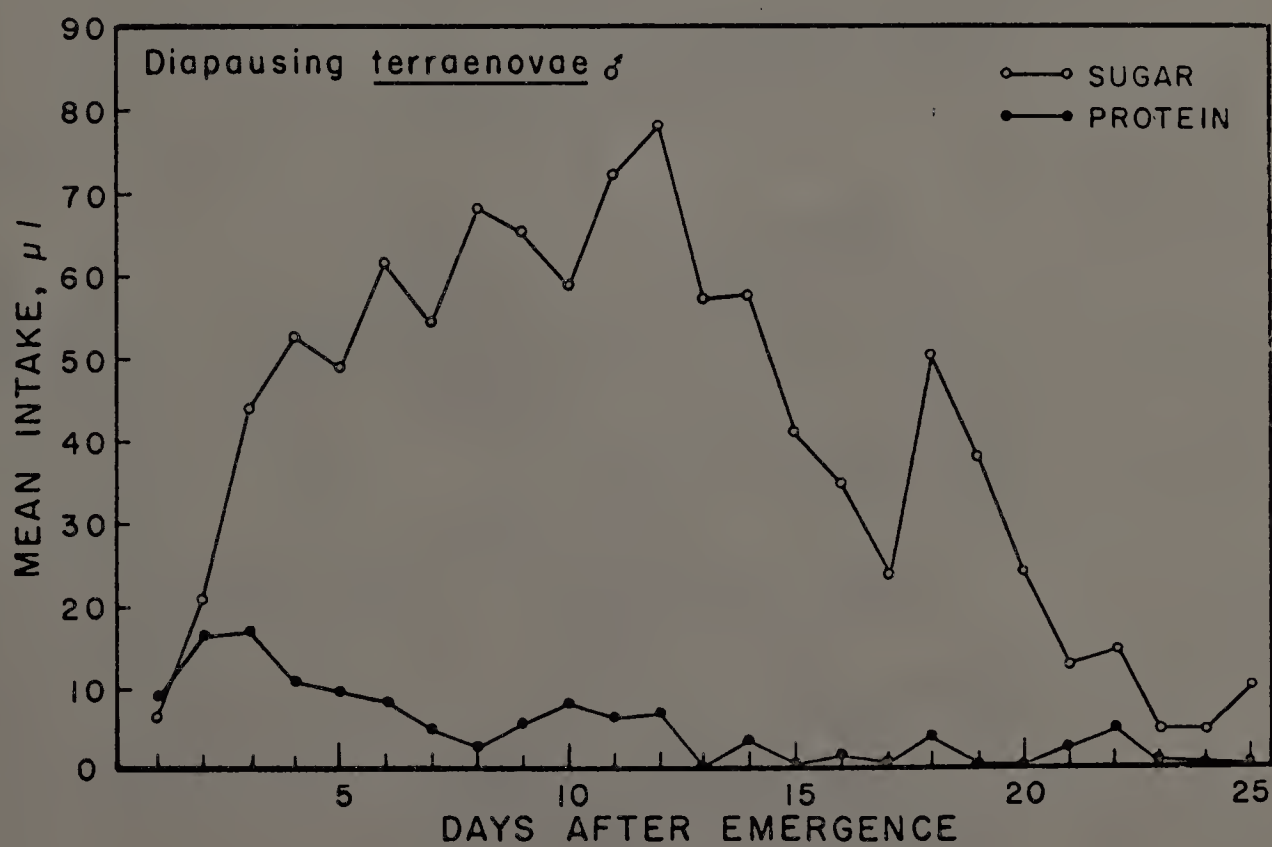
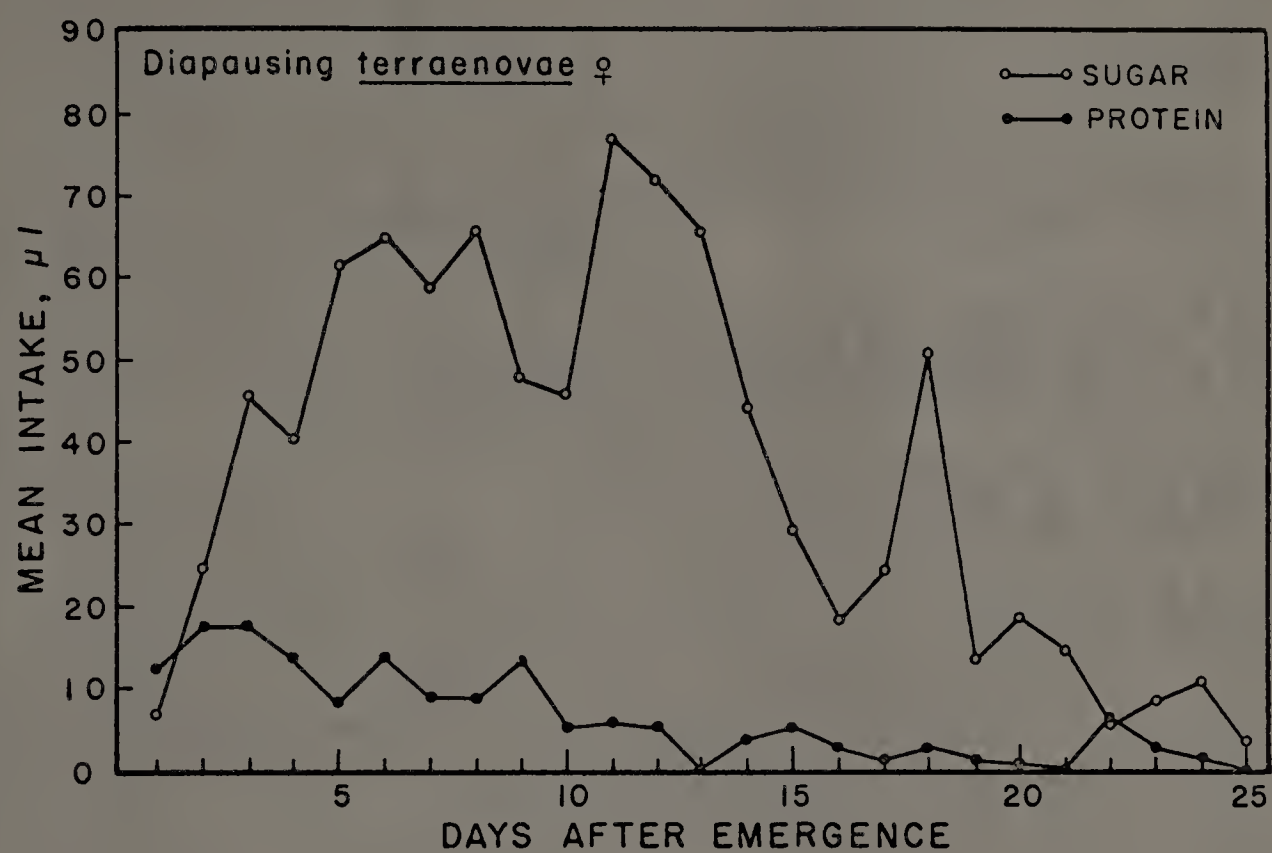


Fig. 6

Table 7. Daily mean intake of protein and sugar by non-diapausing and diapausing P. regina and P. terraenovae.

			DAILY MEAN INTAKE (μ l)		
Experiment			Protein	Sugar	Total
1.	ND, <u>P. regina</u>	Male	4.35	28.24	16.29
		Female	8.98	30.40	19.68
		Male & Female	6.66	29.32	17.99
2.	D, <u>P. regina</u>	Male	2.59	46.34	24.47
		Female	8.07	51.97	30.02
		Male & Female	5.33	49.16	27.18
3.	ND, <u>P. terraenovae</u>	Male	3.65	45.23	24.45
		Female	7.42	47.51	27.47
		Male & Female	5.65	46.26	25.96
4.	D, <u>P. terraenovae</u>	Male	4.36	39.26	21.80
		Female	6.04	36.96	21.50
		Male & Female	5.20	38.11	21.65

ND, Non-diapause; D, Diapause.

DISCUSSION

A. Influence of Age on Intake

The first part of this study was designed to determine the effect of age on the long-term intake of non-diapausing blowflies when given a choice between a protein solution and a sugar solution. The results appear to be consistent with other studies dealing with the intake of protein and sugar by flies (Strangways-Dixon, 1959, 1961; Dethier, 1961; Gelperin and Dethier, 1967; Belzer, 1970).

This study, however, is the first to report on the long-term intake of protein and sugar by both male and female blowflies. It was found that intake was significantly influenced by the age of the fly, which was similar to the findings of Dethier (1961) in a study of the behavioral aspects of protein ingestion, and of Gelperin and Dethier (1967). Gelperin and Dethier (1967), in studies conducted only of males, showed that the intake of .1 M sucrose by male P. regina from day 1 to 60 days after emergence was initially low for the first day or two after emergence. The intake of sucrose increased rapidly on the second day, reached a maximum by the second to fourth day and then gradually declined to the last day of the experiment. Nayar and Sauerman (1974), who studied only females, recently recorded the long-term intake of sucrose by the female mosquito, Aedes taeniorhynchus, and found it to be generally similar to that observed by Gelperin and Dethier (1967) in the blowfly. Intake was initially low but increased and reached a maximum during the first five days and then declined

sharply to a low level which was maintained for the duration of the experiment. It should be noted, however, that Greenberg (1959), in an intake study using male and female houseflies, found no age effect on the intake of protein and sugar. This study was carried out only from day 6 to day 20 after emergence.

The present study showed, furthermore, that the sexes exhibited similar trends with respect to the effect of age on food intake. These results were found to be similar to those of Dethier (1961) who noted that the daily patterns of sugar intake over the lifetime of the fly were similar for both sexes.

A review of the literature on aging in insects (cf. Clark and Rockstein, 1964; Rockstein and Miquel, 1973) reveals that numerous degenerative structural and functional changes occur in adult insects with age. Mechanical "wear and tear" results in damage to the cuticle and wings. There is a progressive loss of flight ability (Williams, et al., 1943; Tribe, 1966), locomotory and mating activities are reduced (Herman, et al., 1971), a general decline is seen in respiratory rates (Calabrese and Stoffolano, 1974a) and in levels of protein synthesis (Levenbook and Krishna, 1971). In addition, degenerative changes have been shown to occur in the number of brain cells and in the fine structure of the central nervous system (Hodges, 1894; Rockstein, 1950; Herman, et al., 1971; Sohal and Sharma, 1972). However, as stated by Gelperin and Dethier (1967), the causes underlying the gradual decline in intake with age are not known. They suggest that this decline could be correlated with decreased activity over the life span, with a gradual accumulation

of stored nutrient reserves or with subtle changes associated with senescence. Nayar and Sauerman (1974), using female mosquitoes, concluded that the decline in intake of sucrose solution with age was correlated with the maximum accumulation of stored energy reserves rather than with decreased activity or subtle changes associated with senescence.

It is most likely that decreased feeding in insects with increasing age is due to several factors, no specific one of which is more important than the other. It is proposed that the decline in intake of sugar and protein by aging flies may be partially the result of reduced energy or carbohydrate requirements resulting from the fact that an old fly is generally less active and flies and mates less than a younger fly. In addition, a decline in fecundity and the number of eggs laid has been shown to occur with increasing age among female insects (Greenberg, 1955; Woke, et al., 1956; Callahan, 1962) which could imply reduced nitrogen requirements with age. This would account for the decline in protein intake also seen with increasing age. However, no clear cut correlation can be found to account for the decline in intake of protein solution by aging male flies.

The decline in intake seen with age in the fly may also be the result of failure of the peripheral chemoreceptor sense organs. These receptors are involved in the initiation of feeding and therefore are vital to the survival of the fly. A degenerated receptor system would imply that aged flies are less efficient feeders since sensory responses from contact chemoreceptors determine

whether or not a medium is ingested. Studies on the effect of age on peripheral sense organs of flies were done by Rees (1970) with P. terraenovae and Stoffolano (1973) with P. regina and showed that among those chemoreceptors that remained functioning, there was a decrease in the mean impulse frequency with increasing age of the fly. Rees (1970) also noted in the same report that the mortality plot for these flies showed a similar time course to the plot of receptor failure and suggested that chemoreceptor failure may be an important factor in determining the life-span of the fly. He further stated that "...it is difficult to understand how a fly could feed at all if there were no peripheral chemosensory drive to initiate the reflex opening of the labellar lobes or the eversion of the proboscis." He concluded that the changes in sensitivity of the receptor cells which appeared to be correlated with age of the adult insect suggest that insect age may need to be considered in a full analysis of feeding behavior in flies.

Intake of protein and sugar. The present study showed clearly that the intake of both protein and sugar by females of both species was greater than that of males. This is in agreement with Greenberg (1959), Dethier (1961) and Belzer (1970) who noted that the volume of protein ingested by females was always greater than that of males.

It is well known that vitellogenesis in female blowflies is dependent on a protein meal (Rasso and Fraenkel, 1954; Harlow, 1956; Orr, 1964a; Belzer, 1970; Bennetová-Rezábová^{V V'}, 1972; Stoffolano, 1974). Furthermore, many authors have reported that spermatogenesis is independent of feeding in P. regina (Cowan, 1932; Mackerras, 1933;

Stoffolano, 1974a) and also in other flies (Chaudhury and Ball, 1973). Thus, it would seem obvious that the protein intake of females would be greater than that of males. In feeding studies, Dethier (1961) and Belzer (1970) noted that an initial peak of protein feeding was found to occur in both sexes following emergence. Females exhibited a second phase of protein consumption which Belzer (1970) termed the "yolk peak" and associated it with vitellogenesis. Following oviposition, protein intake was increased again by females resulting in peaks of protein consumption associated with reproductive cycles (Dethier, 1961; Strangways-Dixon, 1961).

Belzer (1970) noted that there was no tendency towards endogenous cycles of protein consumption in males but found that the first peak of protein consumption exhibited by the male represented the expression of an actual protein preference. One questions as to why males feed on protein at all when spermatogenesis is independent of protein feeding. Stoffolano (1974a) recently demonstrated that a protein meal is necessary for male P. regina to develop the reproductive accessory glands. The secretion from these glands may function to induce oviposition in gravid females and Stoffolano (1974a) has suggested that protein is required to activate the endocrine centers that influence accessory reproductive gland development.

In this study, discrete peaks of protein consumption which could be closely correlated with female reproductive cycles were not found. This observation was thought to be the direct result of the lower temperatures at which these flies were reared as

compared to the temperature at which Belzer (1971) reared his experimental flies. Female P. regina normally oviposit in 5 to 6 days when reared at temperatures around 25 °C. Female P. regina in this study, however, were found to oviposit in 10 to 11 days following emergence at the temperatures (16 h light at 24 °C and 8 h dark at 13 °C) at which they were reared. This delayed oviposition resulted in a depression or flattening out of the curves for protein and sugar intake. Preliminary results of a study which compared the total daily mean intake of protein and sugar by flies reared at 25 °C and another group reared at fluctuating temperatures revealed that the intake by females reared at 25 °C was only slightly more than those reared at the fluctuating temperature regime. Total daily mean intake of protein and sugar expressed in $\mu\text{l}/\text{mg}$ by females reared at 25 °C was 10.52 $\mu\text{l}/\text{mg}$ as compared to 11.63 $\mu\text{l}/\text{mg}$ for flies reared at the fluctuating temperature regime. Females reared at 25 °C oviposited in 6 days while those reared at fluctuating temperatures oviposited in 12 days. These results are consistent with those of Filingier (1931) who studied the effect of temperature on feeding and development of the larvae of Phlyctaenia ferrugalis, the greenhouse leafyter. He found that at 25 °C it took 12.5 days for maturation of 50% of the larvae to the pre-pupal stage while at 20 °C it took 22 days or almost twice as long. However, the total food consumption in mg dry weight by the larvae reared at 20 °C was only slightly more than for those reared at 25 °C. This phenomena had been noted by Rubner in 1908, who states that "...the total energy transformation is

approximately the same regardless of the length of natural life" and is referred to as Rubner's Hypothesis, and is consistent with the existence of a "developmental total" or "thermal constant" common for many processes among poikilothermal animals (Allee, et al., 1949).

In this study, females were also found to have a greater total daily mean intake of sugar than males. Greenberg (1959), however, found no significant difference in sucrose intake for female houseflies as compared to male flies. Calabrese and Stoffolano (1974a) found female P. regina to exhibit higher respiratory rates as compared to male blowflies. They concluded that these differences cannot be evaluated without considering the specific processes which may cause them; such as specific differences in the activity of the reproductive system, locomotory activity, etc.. Norris (1965) and Oldroyd (1964) have suggested that female blowflies are more active than males, the main object of their activity being the location of a suitable oviposition site since food protein is generally more available than oviposition sites (Norris, 1965). In addition, Calabrese and Stoffolano (1974a) noted an increase in respiration rates in sugar fed P. regina compared to females fed on a protein diet and suggested that the need for acquiring protein was expressed as hyperactive behavior and had been made discernable by their respiration studies. Furthermore, it was stated recently by Nayar and Sauerman (1974) that sugar is consumed by female Aedes taeniorhynchus for general maintenance and activity. It therefore does not seem unreasonable to conclude that the greater total intake of sugar by female blowflies is a reflection of their

higher level of locomotory activity and body metabolism. Female blowflies respond to their higher energy needs by ingesting increased amounts of sugar, as compared to males, to replace the reserves used during their activity.

The present study showed that the intake of sugar by female and male blowflies was overwhelmingly greater than protein intake. This finding supports the results of Dethier (1961) who found that if flies had free access to both protein and carbohydrate at all times from the day of emergence, carbohydrate was nearly always taken in greater volume than protein. Dethier (1969) further concludes that the principle food of the blowfly is carbohydrate. We now feel, however, that even though our results are consistent with Dethier, the flies should also be offered plain water in addition to the sugar and protein solutions.

In the present study, sugar intake was seen to fluctuate markedly. These fluctuations were also noted by Dethier (1961) who attributed them to differences in the activity of the fly which was correlated with variations in the climate of the laboratory (temperature and humidity were not held constant). However, Gelperin and Dethier (1967) noted that even when the temperature remains constant, some fluctuation remains. Our results support this and show that even though temperature and humidity were controlled these fluctuations in sugar intake still persisted.

B. Influence of Diapause on Intake

The second part of the present study deals with the question of feeding in diapausing flies. Several excellent discussions of

diapause have been presented and the reader is referred to these for a more complete consideration of diapause in insects (Andrewartha, 1952; Lees, 1955; deWilde, 1962; Danilevskii, 1965; Beck, 1968; Mansingh, 1971). Diapause has been characterized as an endocrine deficiency syndrome (deWilde, 1962) which in adults is manifested by physiological changes such as lack of reproductive development and reduced respiratory rates (Lees, 1956; Slama, 1964; Calabrese and Stoffolano, 1974b). In addition behavioral changes may occur in mating activity (Blondheim and Broza, 1970; ^{VV'}Ždarek, 1970) and flight activity (Nuorteva, 1966; Brown, et al., 1974). Accompanying these various behavioral changes associated with diapause is the observation that many insects show changes in their feeding behavior. However, as stated by Stoffolano (1974b), the extent of feeding prior to, during and after diapause has not been experimentally investigated. This study represents experimental evidence that insects reared in the laboratory under diapause inducing conditions and exhibiting characteristics indicative of imaginal diapause (arrested ovarian development and hypertrophied fat body) do feed on both protein and sugar.

Examination of the literature reveals that many insects have been reported to feed while in a reproductive diapause although this may be limited to non-protein sources and to a "pre-diapause" period of feeding (deWilde, 1954; El-Hariri, 1965; Mitchell and Taft, 1966; Stoffolano, 1968; Hodek, 1971; Brown, et al., 1974). It is the general consensus of these researchers that a period of feeding, prior to an actual period of dormancy is necessary for

the accumulation of large nutrient reserves which are used as an overwintering energy source and thus is essential to the successful survival of the diapausing insect.

The recent reclassification of dormancies in insects and a model put forth by Mansingh (1971) have important application to the question of diapause feeding. In this treatise, diapause is subdivided into three phases; a preparatory period (or pre-diapause period), a diapause period and a post-diapause period. During the pre-diapause period, nutrient food reserves are accumulated as the insect feeds. These reserves are accrued in preparation for the extended period of dormancy which will follow and is an adaptation enabling the insect to survive adverse winter conditions of low temperatures and lack of food. During the diapause period, protein synthesis and metabolism are reduced and stored nutrient reserves are utilized to maintain basal metabolic functions. With the initiation of post-diapause, growth and development are resumed. This description appears to be in general agreement with the observations of feeding during diapause made by the several researchers cited earlier.

Stoffolano (1974b) states that "...it seems obvious that such a period of feeding when large accumulations of food are set aside for the winter should exist", but questions what mechanisms might underlie such behavior. A consideration of possible underlying mechanisms is outside the scope of this discussion. Stoffolano (1968), however, while studying the effect of diapause on the tarsal acceptance threshold of the face fly, Musca autumnalis,

noted a decline in feeding during diapause and proposed an hypothesis to account for this observation. He suggested that the same system of negative feedback operating in the non-diapausing gravid female and also in the non-diapausing male with a distended crop, is operating in diapausing flies to reduce feeding. It was suggested that in the fall, flies enter diapause in response to appropriate environmental stimuli. Flies feed mainly on plant nectar and a period of carbohydrate feeding occurs before fat body hypertrophy results. Stoffolano (1974) extended this hypothesis toward diapause feeding in adult P. regina. It should be noted here that Stoffolano (1968) observed that at 18 °C and a 12 h photophase it took 14 days for adult face flies to develop fat body hypertrophy. It was seen similarly in P. regina and P. terraenovae that fat body hypertrophy was not observed in the diapausing flies for \pm 14 days. As the abdomen of the diapausing fly becomes distended due to fat body hypertrophy and, in the case of P. terraenovae a full crop, body wall stretch receptors may send impulses to the central nervous system via the recurrent nerve, resulting in elevation of the tarsal acceptance threshold and cessation of feeding (Stoffolano, 1968, 1974b).

It is suggested that the decline in intake by diapausing P. regina and P. terraenovae may be the result of a situation similar to that previously described for Musca autumnalis. Both species feed on protein and sugar, although ovarian development is arrested, during a pre-diapause preparatory period which occurs in the late fall when environmental conditions still permit activity and food sources are still abundant. The adult feeds and even engorges on

food, in the case of P. regina, to build up metabolic reserves (such as glycogen and fat resulting in hypertrophied fat body) to be used as an overwintering energy source.

Aging effect during diapause. An important result of the present study was the finding that aging had a statistically greater effect on the intake of the diapausing flies than on the non-diapausing flies. The decline in feeding was coincident with the appearance of fat body hypertrophy in both sexes plus a full crop in P. terraenovae. It is thought that rather than a real aging effect, these other factors resulted in the failure to feed and thus the statistically greater aging effect seen in the diapausing flies as compared to the non-diapausing populations. It should be noted that Stoffolano (1973) measured impulse frequency and determined the number of inoperative chemoreceptor sensilla in diapausing as compared to non-diapausing P. regina. In this study he also found a greater aging effect in the diapausing flies. Sabaratnam (1974) noted that in Calliphora erythrocephala endocrine abnormalities such as seen in flies lacking their medial neurosecretory cells led to an early aging process. Since diapause has been characterized as an endocrine deficiency syndrome, this finding may have important relevance to the observation that intake was more affected by age in the diapausing flies than in the non-diapausing flies.

Intake by diapausing P. regina vs. P. terraenovae. The results of this part of the study on the intake of protein and sugar by diapausing P. regina and P. terraenovae, showed that the feeding patterns of the two species differed in several respects

when compared to each other and to their non-diapausing counterparts. A further difference was seen between the two species of blowflies with respect to the intake of males as compared to females. It is suggested that cessation of feeding due to negative feedback from stretch receptors monitoring abdominal body wall distention may, in part, account for the differences seen in the intake. The enlarged crop seen in P. terraenovae but not in P. regina may have been responsible for the finding that diapausing P. terraenovae feed less than non-diapausing P. terraenovae while the reverse was seen for P. regina. It is possible that negative feedback was less intense in diapausing P. regina and resulted in apparent engorging on sugar by the diapausing as compared to the non-diapausing population.

Since diapause represents an evolved physiological adaptation to overcome adverse environmental conditions of a particular climatic zone (Mansingh, 1971), it seems reasonable that the two species would have evolved different overwintering strategies in response to conditions peculiar to their own ecology. For instance, though both species overwinter as adults, P. regina is a more temperate species in which diapause is temperature dependent while P. terraenovae is an arctic species and diapause appears to be independent of the temperature at photoperiods that would be considered long-day conditions for P. regina. A similar situation was reported to exist for two strains of Chilocorus bipustulatus (Tadmor and Applebaum, 1971) in which one was a southern strain of beetle from Israel and diapauses in response to short photophases and low temperatures while

the other was a more northerly strain from Soviet Central Asia and diapaused at higher temperatures. This is consistent with the view expressed by Danilevskii (1965) that geographical strains adapt their threshold of response to local climatic conditions. Temperatures often rise to above freezing during the winter in temperate regions. Wallis (1962) noted that overwintering P. regina came out of hiding on sunny days during the winter to rest on the sides of buildings. Presumably the extremely low temperatures of the arctic winters would prevent such activity in P. terraenovae. However, our knowledge of the winter biology of the two species is scant and therefore further discussion of evolved differences in overwintering strategies which might explain the observed difference in diapause feeding behavior is limited. Further research on the behavior of these flies in the field is needed.

Protein feeding during diapause. The question of protein feeding during reproductive diapause is an interesting one. Non-diapausing P. regina and P. terraenovae require a protein meal, as already stated, to develop their ovaries. Dethier (1961), Strangways-Dixon (1959, 1961) and Belzer (1970) showed that females selectively increase their protein intake prior to vitellogenesis. During diapause the ovaries do not develop yet in this study it was shown that the quantity of protein intake by diapausing flies as compared to non-diapausing flies was not significantly different (see Table 7) although the diapausing flies did not develop ovaries and instead developed fat hypertrophy. In mosquitoes, the term gonotrophic dissociation (Swellengrebel, 1929) is commonly applied to any

situation where ovaries remain undeveloped in females that have taken a full blood meal (Eldridge, 1966). In such mosquitoes a large fat body develops. The function of ovarian diapause would seem to be to divert nutrients from the ovary to the fat body which serves as a storage depot (Spielman and Wong, 1973).

The question of protein feeding during diapause has been investigated in mosquitoes in studies designed to determine if diapausing mosquitoes taking a late fall blood meal might serve as an overwintering reservoir for viruses (Wallis, 1954; Eldridge, 1966, 1968; Eldridge, et al., 1972; Washino, 1970; Washino, et al., 1971; Spielman and Wong, 1973). It can be stated in summary that with regard to the question of feeding during diapause among overwintering mosquitoes, it seems that fall photoperiods that induce diapause may or may not affect blood feeding. In addition, gonotrophic dissociation is not a general phenomena and, depending on the species, it too may or may not occur among diapausing mosquitoes that take a fall blood meal (Sandburg and Larsen, 1973).

Chen (1969) has suggested that the failure of mosquitoes to take a blood meal in the fall may be the result of changes occurring in the neuroendocrine system in response to a short photophase and low temperature. However, I feel that the mechanism of control of protein feeding during diapause is not simply a neuroendocrine one. I propose the following mechanism to account for the finding of the present study that diapausing flies feed on protein. I suggest that diapause in P. regina and P. terraenovae may occur in three stages as outlined by Mansingh (1971).

The first stage is characterized as a preparatory stage and is accompanied by trophic levels of metabolism and arrested ovarian development. In addition, the corpora allata may be inhibited. Though the insect continues to feed, these nutrients, due to reduced levels of protein synthesis and the occurrence of gonotrophic dissociation, are not metabolized along reproductive pathways but are metabolized instead along diapause pathways. Diapause pathways are accompanied by attenuated levels of digestion and lowered respiration rates and nutrients are accumulated in the fat body. This accumulation of nutrient reserves results in fat body hypertrophy which may act on the central nervous system via stretch receptors to raise the tarsal acceptance threshold and inhibit feeding. During the second stage of diapause, in which the insect ceases feeding, respiration drops to the level of basal metabolism, as described by Slama (1964), and the insect goes into hiding. The first stage would be synchronized to occur with the still favorable conditions existing in the late fall. The insect takes advantage of these conditions to feed and store these reserves to be used later during the second and prolonged stage of diapause which occurs during the severe conditions of low temperatures and lack of food sources associated with winter. The lowered rate of metabolism results in a slow utilization of food reserves during the winter. As the food reserves are utilized, neural inhibition is released and when temperatures again permit activity, the adult emerges in the spring, begins to feed, and the processes of growth and reproduction are resumed.

SUMMARY

1. The long-term intake of protein and sugar by aging and diapausing male and female P. regina and P. terraenovae was measured using a two-choice apparatus.

2. Results of intake by aging P. regina and P. terraenovae were found to be similar; intake was significantly influenced by the age of the fly and was initially low following emergence, increasing rapidly over days 2 to 4 and remaining at a high level (over the first third of the experiment) and then decreasing gradually to the last day of the study. Both sexes showed similar trends (patterns) with respect to age.

3. The cause of the decline in intake was not known, however, it was suggested that failure of peripheral chemoreceptor sense organs may partially contribute to the reduced intake of protein and sugar with age of the fly.

4. The intake of sugar by males and females was significantly greater than protein intake. However, females consumed more than males of both protein and sugar.

5. The occurrence of adult diapause in P. terraenovae appeared to be temperature independent while a low temperature was required for diapause induction in P. regina. Fat hypertrophy did not become apparent in the diapausing flies until about day 14. In addition, the crop of diapausing P. terraenovae was observed to be filled with a clear viscous fluid. This was not observed in P. regina.

6. The intake of both species of diapausing flies was found to be more affected by the age of the fly than in the non-diapausing population. It was suggested that, rather than a real aging effect, the occurrence of fat body hypertrophy in both species and a full crop in P. terraenovae resulted in the failure to feed and thus the statistically greater aging effect in the diapausing populations.

7. Diapausing P. regina fed significantly more on sugar than the non-diapausing population while the reverse was seen for the diapausing P. terraenovae. These differences in feeding patterns of the two diapausing species was thought to represent differences characteristic of the species and their respective geographical adaptations.

8. Protein intake by the non-diapausing flies as compared to the diapausing flies of both species was not significantly different. The intake of sugar by the diapausing males and females was significantly greater than protein intake. An hypothesis is put forth to account for the occurrence of protein and sugar feeding by diapausing flies.

APPENDIX

Table 1. Analysis of variance of daily intake of protein and sugar by non-diapausing male and female Phormia regina from day 1 to 40 days after emergence.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	1284084.9700	1284084.9700	
Sex(S)	1	3217.4490	3217.4490	9.2449**
Age(A)	39	101099.0126	2592.2824	7.4485**
Diet(D)	1	122924.9652	122924.9652	353.2094**
SA	39	12672.1540	324.9270	.9336
SD	1	424.3010	424.3010	1.2191
AD	39	42854.9592	1098.8451	3.1573**
SAD	39	14472.8351	371.0983	1.0663
R(SAD)	9606	334101.9750	348.0229	

Table 2. Analysis of variance of daily intake of protein and sugar by diapausing male and female Phormia regina from day 1 to 40 days after emergence.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	2222693.9490	2222693.9490	
Sex(S)	1	8636.8033	8636.8033	43.3772**
Age(A)	39	86292.7780	2212.6353	11.1126**
Diet(D)	1	487096.8882	487096.8882	2446.3843**
SA	39	12459.2451	319.4678	1.6044**
SD	1	1.5022	1.5022	4.0075**
AD	39	38096.7217	976.8390	4.9060**
SAD	39	11664.3135	299.0850	1.5021*
R(SAD)	960	191144.5365	199.1089	

Table 3. Analysis of variance comparing daily intake of protein and sugar by non-diapausing versus diapausing male and female Phormia regina.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	3437090.7324	3437090.7324	
Treatment(T)	1	63196.0144	63196.0144	232.3061**
Sex(S)	1	10874.8895	10874.8895	39.9756**
Age(A)	39	157706.7658	4043.7632	14.8647**
Diet(D)	1	547425.1381	547425.1381	2012.3142**
TS	1	579.1206	579.1206	2.1288
TA	39	30205.5696	774.5081	2.8476**
SA	39	12579.4385	322.5497	1.1856
TD	1	59560.0505	59560.0505	218.9450**
SD	1	232.2356	232.2356	.8537
AD	39	60551.6443	1552.6063	5.7073**
TSA	39	12095.8095	310.1490	1.1400
TSD	1	192.9699	192.9699	.7093
TAD	39	19661.0080	504.1284	1.8531**
SAD	39	15020.1646	385.1324	1.4157*
TSAD	39	10532.4469	270.0627	.9927
R(TSAD)	1920	522312.2104	272.0376	

Table 4. Analysis of variance of daily intake of protein and sugar by non-diapausing male and female Protophormia terraenovae from day 1 to 25 days after emergence.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	1460258.9830	1460258.9830	
Sex(S)	1	1597.4293	1597.4293	5.6222*
Age(A)	24	45057.6149	1877.4006	6.6076**
Diet(D)	1	290186.6010	290186.6010	1021.3321**
SA	24	8978.6636	374.1110	1.3167
SD	1	97.9206	97.9206	.3446
AD	24	62240.5223	2593.3551	9.1274**
SAD	24	8766.0683	365.2528	1.2855
R(SAD)	600	170475.3652	284.1256	

Table 5. Analysis of variance of daily intake of protein and sugar by diapausing male and female Protophormia terraenovae from day 1 to 25 days after emergence.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	1143373.4902	1143373.4902	
Sex(S)	1	16.6074	16.6074	.1201
Age(A)	24	100500.9521	4187.5397	30.3087**
Diet(D)	1	181901.3041	181901.3041	1316.2613**
SA	24	3247.4840	135.3118	.9738
SD	1	689.2812	689.2812	4.9877*
AD	24	80223.3610	3342.6400	24.1877**
SAD	24	4143.8353	172.6598	1.2493
R(SAD)	600	.82916.9818	138.1950	

Table 6. Analysis of variance comparing daily intake of protein and sugar by non-diapausing versus diapausing male and female Protophormia terraenovae.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	2593954.5457	2593954.5457	
Treatment	1	9677.9274	9677.9274	45.8321**
Sex	1	644.1408	644.1408	3.0504
Age	24	125590.9661	5232.9569	24.7836**
Diet	1	465794.5155	465794.5155	2205.8811**
TS	1	969.8959	969.8959	4.5931*
TA	24	19967.6009	831.9834	3.9400**
SA	24	4439.4000	184.9750	.8759
TD	1	6293.3896	6293.3896	29.8038**
SD	1	653.3986	653.3986	3.0943
AD	24	126528.0579	5272.0024	24.9668**
TSA	24	7786.7476	324.4478	1.5364*
TSD	1	133.8032	133.8032	.6336
TAD	24	15935.8254	663.9927	3.1444**
SAD	24	5550.3393	231.2641	1.0952
TSAD	24	7359.5643	306.6485	1.4522
R(TSAD)	1200	253392.3471	211.1603	

Evaporation controls. An analysis of variance (Tables 8 and 9) was made of data obtained from daily measurements of evaporation among protein and sugar controls in both non-diapausing and diapausing experiments. Since the results of the diapausing and non-diapausing experiments with the two species of blowflies were not compared statistically, evaporation data for these two species were analyzed separately. These data are summarized in Table 10. Comparisons of total daily mean evaporation between non-diapausing and diapausing P. regina controls showed a significant difference at the $P = .01$ level (Table 8). Total daily evaporation for the non-diapausing controls was 15.87 μl as compared to 17.30 μl for the diapausing controls. However, an analysis of Treatment X Diet showed no significant interaction. Total daily mean evaporation of protein for the non-diapausing P. regina was 16.72 μl as compared to 15.01 μl for sugar while total daily mean evaporation of protein for the diapausing P. regina was 18.36 μl as compared to 16.24 μl for sugar.

Analysis of Treatment X Age showed a significant interaction ($P < .01$); evaporation among non-diapausing controls ranged from a low of 13.59 μl on day 2 to a high of 17.84 μl on day 17 while evaporation among the diapausing controls ranged from a low of 12.75 μl on day 37 to a high of 22.80 μl on day 24. There was no significant Age X Diet interaction, however, some interaction was seen among Treatment X Age X Diet effects.

Comparisons of total daily mean evaporation between controls of non-diapausing and diapausing P. terraenovae showed a significant

difference ($P < .01$, Table 9). Total daily mean evaporation for the non-diapausing controls was 19.17 μl as compared to 18.76 μl for the diapausing controls. There was a small Treatment X Diet interaction ($P < .05$). Total daily mean evaporation of protein for the non-diapausing P. terraenovae was 19.66 μl as compared to 19.77 μl for sugar while total daily mean evaporation of protein for the diapausing P. terraenovae was 19.09 μl as compared to 18.42 μl for sugar.

There was a slight Treatment X Age interaction ($P < .05$). Total daily mean evaporation for the non-diapausing population ranged from a low of 17.25 μl on day 16 to a high of 22.39 μl on day 1. Total daily mean evaporation for the diapausing controls ranged from a low of 15.98 μl on day 16 to a high of 20.90 μl on day 18. There was no Age X Diet or Treatment X Age X Diet interaction.

Table 8. Analysis of variance comparing daily evaporation of protein and sugar in non-diapausing and diapausing experiments with Phormia regina.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	308262.7716	308262.7716	
Treatment(T)	1	574.3752	574.3752	63.0821**
Age(A)	39	2392.9638	61.3580	6.7387**
Diet(D)	1	1026.4783	1026.4783	112.7353**
TA	39	1636.7932	41.9691	4.6093**
TD	1	11.6321	11.6321	1.2775
AD	39	486.2632	12.4683	1.3693
TAD	39	539.5479	13.8346	1.5194*
R(TAD)	960	8741.0127	9.1052	

Table 9. Analysis of variance comparing daily evaporation of protein and sugar in non-diapausing and diapausing experiments with Protophormia terraenovae.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Mean	1	259139.7121	259139.7121	
Treatment(T)	1	159.4802	159.4802	28.1344**
Age(A)	24	799.8870	733.3286	5.8797**
Diet(D)	1	13.6529	13.6529	2.4085
TA	24	257.1320	10.7138	1.8900**
TD	1	26.8442	26.8442	4.7356*
AD	24	183.2335	7.6347	1.3468
TAD	24	190.6510	7.9438	1.4013
R(TAD)	600	3401.1269	5.6685	

Table 10. Analysis of variance of protein and sugar by non-diapausing and diapausing controls of P. regina and P. terraenovae.

DAILY MEAN EVAPORATION (μ l)			
Experiment	Protein	Sugar	Total
1. ND, <u>P. regina</u>	16.77	15.01	15.87
2. D, <u>P. regina</u>	18.36	16.24	17.30
3. ND, <u>P. terraenovae</u>	19.66	19.77	19.17
4. D, <u>P. terraenovae</u>	19.09	18.42	18.76

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