BIOCHEMICAL CHANGES IN THE CROP, OESOPHAGUS AND POSTPHARYNGEAL GLAND OF COLONY-FOUNDING RED IMPORTED FIRE ANT QUEENS (*SOLENOPSIS INVICTA*)

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Abstract—Chemical analysis of hydrocarbons, triacylglycerols and free fatty acids present in hexane extracts of the crop, oesophagus and postpharyngeal gland of colony-founding queens of *Solenopsis invicta* Buren illustrates that the oesophagus enlarges into a thoracic crop concomitant with wing muscle histolysis. The flow of material goes from the crop to the oesophagus, but not to the postpharyngeal gland. In the crop and oesophagus triacylglycerols are the dominant chemical class, whereas the postpharyngeal gland contains primarily hydrocarbons. The pattern of postpharyngeal gland hydrocarbons changes between the time of insemination and 25 days after mating.

Key Word Index: Postpharyngeal gland, crop, oesophagus, thoracic crop, *Solenopsis invicta* queens, triacylglycerols, hydrocarbons, free fatty acids

INTRODUCTION

Wing muscle histolysis in colony-founding ant queens was reported by Janet (1907). The phenomenon is generally coordinated with mating and brood development, with the histolysis products reportedly used for self-maintenance and for sustaining the first brood (Wilson, 1971). Similarly, newly mated *Solenopsis invicta* Buren queens undergo dealation, which triggers numerous physiological changes including wing muscle histolysis (Markin et al., 1972). The released glycogen, protein and amino acids were suggested to be utilized for ovariole maturation and the queen's maintenance during the critical time period between mating and the emergence of the first workers (Markin et al., 1972; Toom et al., 1976a, b). This event takes on an added dimension when coupled with a report by Petersen-Braun and Buschinger (1975) that queen ants of five genera of two subfamilies develop a thoracic crop formed by the expansion of the oesophagus into the void left in the thorax after wing muscle histolysis. The second food storage vesicle is necessary, according to Petersen-Braun and Buschinger (1975), because ovariole development in the abdomen is so great that the gastral crop cannot expand as in uninseminated female alates. *Solenopsis invicta* also has a highly pleated tube-like oesophagus that expands with fluid after wing muscle histolysis to occupy the entire thoracic cavity (Glancey et al., 1980).

The situation of food storage and utilization is further complicated by the report that the post-pharyngeal gland in *S. invicta* appears to function as a gastric caecum (Vinson et al., 1980). Compared to workers the gland is more highly developed in fire ant

queens, in which the multilobed structure occupies a large portion of the head capsule (Phillips and Vinson, 1980a). The 10-fold increase of the gland weight per unit body length compared with that of minor workers suggests a special function for the gland in queens.

Usually the content of the crop and postpharyngeal gland are simply described as a yellowish oil. Very few chemical analyses have been reported on the contents of the postpharyngeal gland. Barbier and Delage (1967) found fatty acids and sterols in the postpharyngeal gland of *Messor capitatus* Latr. Paulsen (1969) found free fatty acids and triacylglycerols in *Formica polyctena* Foerst and Peregrine et al. (1973) found ergosterol in glands from *Acromyrmex octospinosus* Reich alates.

This report presents data on the changes that occur with increasing age after mating in hydrocarbons, triacylglycerols and free fatty acids in the crop, oesophagus and postpharyngeal glands of colony-founding *Solenopsis invicta* queens. The results clearly show thoracic crop development and the dynamic changes that occur in the postpharyngeal gland.

MATERIALS AND METHODS

Newly mated queens were collected from Gainesville parking lots or other convenient surfaces immediately after mating flights. They were placed individually in small plastic cups with moistened plaster of Paris bottoms. After the minims (first workers produced by a colony-founding queen) eclosed, the colonies were transferred to test tubes in small trays (Banks et al., 1981) and fed as described by Williams et al. (1980).

Five individual replicate specimens were taken at 0, 5, 10, 15, 25 and 120 days after mating. The queens were held in the laboratory at 27°C. The crop, oesophagus and post-pharyngeal gland of each were extirpated and the intact glands were broken in 20 μl of hexane with a dissection probe.

The hexane was removed and the parts to be analyzed were washed twice with 50 μl hexane. The combined hexane extracts were used for subsequent analyses.

Hydrocarbons. triacylglycerols and free fatty acids were separated and isolated by preparative thin layer chromatography (Whatman LK6D Linear-K [*] silica gel 250 μ, or Whatman PLK5F Linear K [*] silica gel 1000 μ). Standards were spotted at each end of the plates. The plates were developed with hexane. diethyl ether, formic acid (80:20:2 by vol.) and the positions of the lipids visualized by spraying with 0.1% 2',7'-dichlorofluorescein in methanol. After drying. dark spots could be seen on a yellow-green background under u.v. light. Spots corresponding to diacylglycerols. sterols and monoacylglycerols were observed but not investigated. Individual extracts within an age group were visually compared for homogeneity and samples of crops, oesophagi and postpharyngeal glands from the same queens were directly compared by TLC on a qualitative and semi-quantitative basis by observing the intensity of the visualized lipids. Hydrocarbons from each crop and postpharyngeal gland extract were collected separately, whereas those from the oesophagus were combined for each time period. An n-pentacosane standard (Applied Science Laboratories) was added to each sample and used to provide quantitative data for the hydrocarbons. The triacylglycerols and free fatty acids were combined for each collection time in all cases. Hydrocarbons were analyzed by gas chromatography (GC) on a Varian 3700 [*] equipped with a flame ionization detector using 3% (w/w) OV-101 and 3% (w/w) OV-17 on 100/120 or 120/140 Gas Chrom Q [*] packed in 1.8 m × 2 mm i.d. glass columns. Qualitative and quantitative data were obtained with a Hewlett Packard model 3385A [*] data processor. Internal standards triheptadecoin and heptadecanoic acid (Applied Science Laboratories) were added to the isolated triacylglycerols and free fatty acids. respectively. The samples were then transesterified or esterified by heating at 90°C with 4% (v/v) H₂SO₄ in methanol for 2 hr in a reacti-vial (JOHNSTON, 1971). The isolated products were analyzed by gas chromatography using 10% (w/w) Silar 10C [*] on 100/120 Gas Chrom Q [*] and 3% (w/w) OV-17 [*] packed in 1.8 m × 2 mm i.d. glass columns. Quantitative data were collected using internal standards (external standards gave similar results) and peak identification was made by direct comparison with standard fatty acid methyl esters (Applied Science Laboratories).

RESULTS

Quantitative

The crop is characterized by having large amounts of triacylglycerols at day 0 (Fig. 1). which decline rapidly during the time of first brood development to the lowest value obtained at 25 days after mating. The gastric crop did not regain the original triacylglycerol level even after workers became available to feed and care for the queen. Hydrocarbons and free fatty acids remained at low levels compared to triacylglycerols throughout the 120-day test period.

The oesophagus data (Fig. 2) show a rapid increase in triacylglycerol levels starting almost immediately after mating. Triacylglycerols reached a maximum at the 15-day sample and then declined rapidly to the lowest value obtained at day 25, corresponding to wing muscle histolysis. which was complete by day 15 and first brood development. Both hydrocarbons and free fatty acids increased from trace amounts at day 0 to measurable but low levels throughout the remainder of the test period.

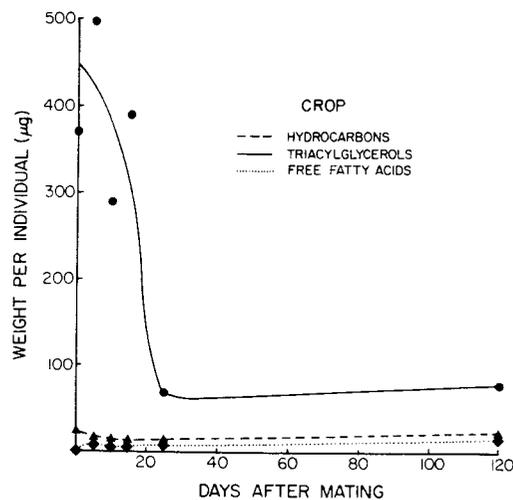Unlike the crop and oesophagus. the post-

Fig. 1. Quantitative changes in hydrocarbons, triacylglycerols and free fatty acids in the crop of colony-founding *S. invicta* queens with age after mating. Points represent values obtained from an average of five separate samples for hydrocarbons and one fifth of a pooled sample from five individuals for triacylglycerols and free fatty acids.
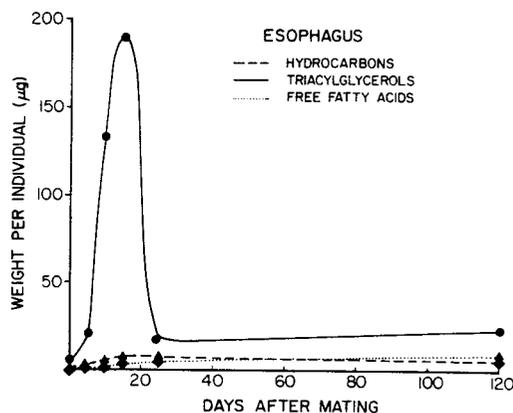

Fig. 2. Quantitative changes in hydrocarbons, triacylglycerols and free fatty acids in the oesophagus of colony-founding *S. invicta* queens with age after mating. Points represent values obtained as detailed in Fig. 1.

pharyngeal gland extracts are dominated by hydrocarbons (Fig. 3). There is a rapid increase in hydrocarbons between days 10 and 15, again corresponding to both wing muscle histolysis and first brood development. The hydrocarbon levels decreased after 15 days to a level similar to that of the day 0 newly mated queens. Triacylglycerols and free fatty acids remained at low levels during the test period.

Qualitative

The methyl esters derived from transesterification of crop triacylglycerols were dominated by oleic acid and palmitic acid (Table 1). The mean ratio ± S.D. of unsaturated to saturated esters (U/S) obtained for the six age groups of the test period was 1.71 ± 0.13. This ratio and the triacylglycerol composition was
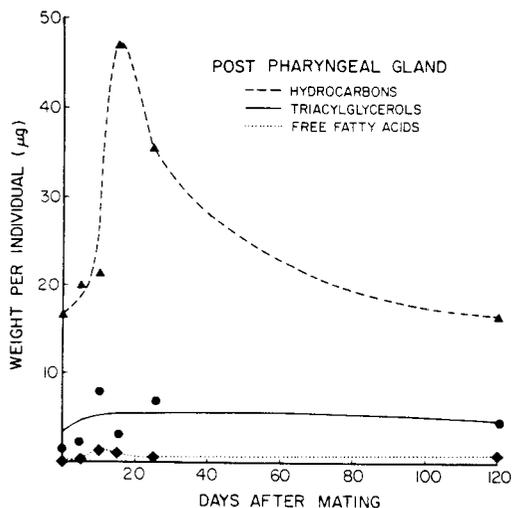
Fig. 3. Quantitative changes in hydrocarbons, triacylglycerols and free fatty acids in the postpharyngeal gland of colony-founding *S. invicta* queens with age after mating. Points represent values obtained as detailed in Fig. 1.

Table 1. Mean percent triacylglycerol fatty acid ester composition from the crop, oesophagus and postpharyngeal gland of colony founding *S. invicta* queens ($n = 6$)

Fatty acid ester	Crop (S.E.M.)	Oesophagus (S.E.M.)	Postpharyngeal gland (S.E.M.)
14:0	4.2 (0.5)	4.4 (1.7)	8.0 (0.8)
16:0	22.3 (0.6)	23.1 (0.5)	25.7 (0.9)
16:1	7.3 (0.5)	7.5 (1.7)	4.6 (1.7)
18:0	9.3 (0.5)	8.8 (0.5)	11.2 (0.3)
18:1	42.8 (0.8)	43.4 (1.3)	36.8 (3.3)
18:2	9.9 (0.4)	10.0 (0.7)	8.6 (1.8)
18:3	3.1 (0.8)	2.6 (0.7)	4.9 (1.3)

remarkably consistent throughout the test period. The methyl esters of the crop free fatty acids showed a quantitatively more variable but qualitatively similar pattern (U/S ± S.D. = 2.70 ± 1.60). Crop hydrocarbons were dominated by five major components with GC retention times from nC_{27} to nC_{28} paraffins (Fig. 4A). The compounds have been identified by Nelson *et al.* (1980) from *S. invicta* cuticular hydrocarbons and by Thompson *et al.* (1981) from queen postpharyngeal glands as *n*-heptacosane, 13-methylheptacosane, 3-methylheptacosane, 13,15-dimethylheptacosane and 3,9-dimethylheptacosane. Gas chromatography using OV-101 as the stationary phase gave only four major peaks due to the co-elution of 3-methylheptacosane and 13,15-dimethylheptacosane. Use of OV-17 effected an adequate separation of the five major components. The relative percentages of the major components were similar throughout the test period.

The pattern of triacylglycerol fatty acid methyl esters of the oesophagus directly paralleled those of the crop as shown in Table 1. Similarly, the free fatty acids and hydrocarbons were qualitatively identical to those found in the crop. Direct visual comparison of the crop and oesophagus contents of the same individuals on TLC showed the complex pattern and relative spot intensities of hydrocarbons, triacylglycerols, free fatty acids, diacyglycerols, sterols and monoacylglycerols to be identical.

The triacylglycerol fatty acid methyl esters of the postpharyngeal gland (Table 1) were qualitatively more variable from sample to sample than those of the crop and oesophagus and were characterized by a lower mean ratio ± S.D. of unsaturated to saturated fatty acids (1.16 ± 0.20). The free fatty acid composition was also variable and had an even lower unsaturation ratio ± S.D. (0.87 ± 0.33). The postpharyngeal gland hydrocarbons had a pattern of five major components at day 0, similar to the crop (Fig. 4A), but by day 25 some of the individual samples showed dramatic changes in the relative amounts of *n*-heptacosane and 13,15-dimethylheptacosane (Fig. 4B). By 120 days after mating all extracts showed the virtual absence of *n*-heptacosane and 13,15-dimethylheptacosane. These changes were first noticed in day 25 samples, but they were probably in
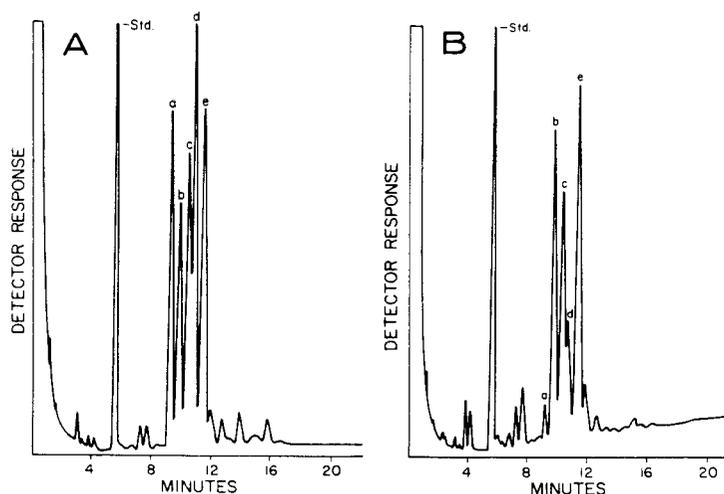

Fig. 4. Gas chromatography trace of (A) crop hydrocarbons (B) postpharyngeal gland hydrocarbons 120 days after mating. a. heptacosane; b. 13-methylheptacosane; c. 3-methylheptacosane; d. 13,15-dimethyl-heptacosane; e. 3,9-dimethylheptacosane (3% OV-17 on 120/140 Gas Chrom Q. 200–250 C min).

progress during the intense biosynthetic period between 10 and 15 days after mating (Fig. 3).

DISCUSSION

The quantitative results for triacylglycerols in the crop and oesophagus (Figs. 1 and 2) clearly illustrate the formation of a thoracic crop (GLANCEY *et al.*, 1980 and PETERSEN-BRAUN and BUSCHINGER, 1975). The qualitative analysis of each chemical class unambiguously shows that the thoracic crop extract is identical to that found in the gastral crop extract. The low levels of hydrocarbon and free fatty acids in the crop and oesophagus extracts do not change significantly when compared to the changes that occur in triacylglycerols. It is, therefore, very likely that the majority of these hydrocarbons and free fatty acids are derived from the tissues themselves and not from the gland or organ contents. The weight of the crop minus hexane extractable lipids is the same for queens caught on their nuptial flight and established queens. However, the oesophagus doubles in weight (VINSON *et al.*, 1980). These data are compatible with our results. The decline in amounts of triacylglycerols in the crop and the corresponding increase in triacylglycerols in the oesophagus began immediately after mating and continued until 15 days after mating. This indicates that the process occurs concomitant with wing muscle histolysis and that the development and filling of the thoracic crop occurs at a faster rate than the contents are consumed by early instar larvae and utilized by the queen. After approximately 15 days the nutritional requirements of the queen and brood increase to the point where the loss of food material through the crop and thoracic crop was very rapid (Figs. 1 and 2) and consequently by day 25 the amount of stored triacylglycerols decreased to a minimum. Even after 120 days queens do not significantly utilize their crop or thoracic crop for food storage, although they no longer must feed their brood and are being fed by workers. Their own nutritional requirements for egg production and self maintenance must preclude the accumulation and storage of triacylglycerols. In fact, older colony queens have never been found with filled crops or distended oesophagi (GLANCEY *et al.*, 1980). Therefore it appears that the primary function of the gastral crop in *S. invicta* queens is to ensure successful colony founding by having the necessary self-contained food stores available for first brood development.

PETERSEN-BRAUN and BUSCHINGER (1975) suggested that the formation of a thoracic crop was due to the displacement of crop contents by the rapid ovariole development that occurs after mating. This may be partly true with *S. invicta*; however, GLANCEY (unpublished results) has noted that ovariole and thoracic crop development do not always occur simultaneously. It follows then that thoracic crop formation is directly linked with wing muscle histolysis and can occur independently of ovariole development. The crop of female alates can be likened to a balloon that expands with pressure as it fills with triacylglycerols. Newly-mated queens alight from the mating flights with a full crop that readily releases its contents into the oesophagus as the musculature in the thorax breaks down.

The source of postpharyngeal gland contents and their function have been the subject of numerous reports. BUGNION (1930) suggested that the postpharyngeal gland plays a role in larval feeding, while FORBES and McFARLANE (1961) speculated that the gland serves the digestive activities of the individual ant. Although lipase activity has been found in the postpharyngeal gland of several ant species (AYRE, 1967; RICKS and VINSON, 1972), the level of activity is very low and does not support involvement of the gland in lipid digestion. Also, DELAGE-DARCHEN (1976) showed that at least part of the lipase is absorbed into the postpharyngeal gland with food. ZYLBERBERG *et al.* (1974) investigated the postpharyngeal gland morphology of several ant genera and concluded that the glands absorb lipids from ingested food. Similarly, PEREGRINE and MUDD (1974) showed that the colour difference in the contents of worker and alate postpharyngeal glands of *Acromyrmex octospinosus* was due to a difference in food intake. MARKIN (1970) suggested that the contents may be a mixture of secretory products from the glandular cells and lipids from ingested foods. He went on to show that the postpharyngeal gland contents of *Iridomyrmex humilus* (Mayr) were fed primarily to the queen and larvae. BRIAN and BLUM (1969) found that fatty acids from the heads of *Myrmica rubra* (L.) queens had an effect on larval growth. They postulated that the acids came from either the mandibular or postpharyngeal glands. BRIAN (1968) suggested that primer pheromones may accumulate in the postpharyngeal gland lumen of *M. rubra* workers due to their tending activities. VINSON *et al.* (1980), using radiolabelled triolein, claimed that lipids in the postpharyngeal gland of red imported fire ants are sequestered from the lumen by gland cells and transported into the haemolymph. However, the postpharyngeal glands of queens did not sequester triacylglycerols or fatty acids from the haemolymph (PHILLIPS and VINSON, 1980b). Their overall conclusions are that the gland functions as a cephalic caecum and that the major lipid components come from the food of the adult ant. However, THOMPSON *et al.* (1981) discovered that the major class of compound in the postpharyngeal glands of *S. invicta* queens is hydrocarbon composed almost entirely of four uncommon methyl-branched compounds. VANDER MEER (unpublished results) has shown the species specificity of these compounds and that in female alates they are not derived from exogenous food sources. Gas chromatographic analysis of newly-mated queen postpharyngeal gland contents, obtained using a microcapillary tube, gave the characteristic pattern of hydrocarbons shown in Fig. 4A, proving that the hydrocarbons are not only associated with glandular tissue. Our data show that the postpharyngeal gland does not function solely as a cephalic caecum, since its contents do not mirror those found in the crop or later in the thoracic crop. The dominance of hydrocarbons and the qualitative consistency of the hydrocarbon pattern for each time period and individual specimen also precludes their being derived from an exogenous food source. The marked increase in hydrocarbon at 15 days after mating (Fig. 3)

suggests that the queen has the biosynthetic capacity to produce these materials herself and that possible transfer of hydrocarbons from workers is not involved. This peak of hydrocarbon biosynthesis may represent an altered biochemical path as evidenced by the intriguing change in hydrocarbon pattern that first clearly appears at 25 days after mating and is complete within 120 days (Fig. 4A and 4B). This time-dependent change also indicates that the postpharyngeal gland contents are not static but undergo a continuous turnover, at least in young queens. Eggs, larvae and queen parts (from alate or established functional queens) such as the ovarioles, crop and cuticle do not change their hydrocarbon composition and have a pattern similar to Fig. 4A and not Fig. 4B (VANDER MEER, unpublished results). It would appear that the postpharyngeal gland contents are being released to larvae or workers. These facts coupled with PHILLIPS' and VINSON's (1980a) observation that the postpharyngeal gland is most highly developed in queens indicate special unidentified functions for the postpharyngeal gland in *S. invicta* queens. THOMPSON *et al.* (1981) suggested that the postpharyngeal gland may be important in overall colony organization, caste determination and/or food exchange or queen and brood tending. Actual proof of these functions awaits the development of appropriate bioassays.

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