RAPID DETERMINATION OF TOTAL LIPIDS IN MOSQUITOES[1]

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ABSTRACT. Total lipids in individual mosquitoes can be determined by extraction with chloroform-methanol followed by reaction with sulfuric acid and a vanillin-phosphoric acid reagent. By subtracting the lipids determined in a starved population, the method is suitable to establish variations of lipid reserves in field populations.

INTRODUCTION

Female mosquitoes supported by stored lipids can survive a long time, resting on the ground or under dense foliage, in spite of unfavorable nutritional and climatic conditions. The physiology of lipids in mosquitoes has been studied in the laboratory (Van Handel 1984), but there are no data on variability of the lipid reserves for mosquitoes caught in the field under different climatic conditions and with attractant and nonattractant trapping methods. We have begun to fill this gap (Day and Van Handel, personal communication) supported by a method that makes possible the analysis of several hundred individual mosquitoes per day.

To the insect biochemist, a variety of techniques such as thin layer, gas and high pressure liquid chromatography, are available to separate and identify phospholipids, fatty acids, cuticular waxes, sterols, water-insoluble hormones and pheromones (Jackson and Armold 1977). To the field entomologist who seeks correlations between available lipid reserves and longevity or behavior, the choice is limited.

Extraction in a soxhlet apparatus with light petroleum or methylene chloride followed by gravimetrical assay is cumbersome and is not suitable for the small amount of lipids found in individual mosquitoes. Chloroform-methanol is a much more efficient and widely used analytical lipid solvent. The disadvantage is that the methanol in combination with tissue water also extracts non-lipid material such as glycerol, sugars, amino acids and some peptides. This requires re-extraction with water to remove this non-lipid material or the use of a colorimetric assay that is not sensitive to these impurities.

Clinical laboratories use a highly specific enzymatic assay (available in kits) for serum triglycerides (the main component of metabolizable lipids), but it can only be used for the extracellular water-soluble lipoprotein complexes of serum and not for intracellular water-insoluble fat droplets that form the bulk of insect lipid reserves. A chemical assay (now largely abandoned in favor of the enzymatic triglyceride assay) that reacts with all types of lipids but with none of the above-mentioned impurities has been used in German clinical laboratories. It can be applied to serum without previous extraction and has been used to monitor hemolymph lipids during isolation and purification of a lipid-regulating hormone from the locust corpus cardiacum (Holwerda et al. 1977). It consists of treatment with hot sulfuric acid that converts unsaturated lipids to water-soluble sulfonic acid derivatives. These develop a reddish color after addition of a vanillin-phosphoric acid reagent (Zöllner and Kirsch 1962). In combination with a simple extraction with methanol-chloroform, it has the sensitivity, specificity and speed to survey the level of metabolizable lipids in individual mosquitoes collected in the field. Assay of a population starved to death provides a baseline that includes all non-metabolizable lipids.

MATERIALS

Vanillin-phosphoric acid reagent. Dissolve 600 mg vanillin in 100 ml hot water. Add 400 ml 85% phosphoric acid and store in dark. The reagent is stable for several months, but should be discarded when it darkens.

Sulfuric acid (95–98%).

Chloroform-methanol (1:1)

Lipid standard: 100 mg per 100 ml of a commercial vegetable oil (*e.g.*, soy bean oil) in chloroform.

16 × 100 mm culture tubes. Marked at 5 ml level.

An aluminum block, provided with 17 × 80 mm holes to accommodate the culture tube is mounted on a laboratory hot plate kept at approximately 100°C. A thermometer in a tube containing a few ml glycerol is placed in the heating block to allow temperature monitoring. Temperature is not crucial and may vary between 90 and 110°C.

Field collected insects can be stored for later analysis at −20°C or colder. Alternatively, mosquitoes may be heated to constant weight at 90°C (about 1 hour). This inactivates enzymes

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that may cause *post mortem* changes and also preserves carbohydrates.

PROCEDURE

Place a mosquito into a tube and crush with a glass rod in about 0.5 ml of chloroform-methanol. Gently shake the tube to include mosquito parts on sides, then carefully transfer supernatant to a clean tube. Traces of mosquito tissue transferred with the solvent do not interfere and centrifugation is unnecessary.

Place tubes in heating block to evaporate the solvent. Add 0.2 ml of sulfuric acid, and heat for 10 min. After cooling, pour vanillin reagent from a beaker to the 5 ml mark, mix, and allow the reddish color to develop for at least 5 min. The color is stable from 5 to 30 min, then slowly fades. The tubes are read directly in a spectrophotometer at 525 nm against a reagent blank. Optical densities higher than 1.00 can be re-read against the blank and the standards at 490 nm, where the value is about 50% of that at 525 nm. Alternatively, one ml of the high density solution can be transferred with a glass syringe to a new tube and filled to the 5 ml mark with the vanillin reagent. The original optical density is 5X the diluted value.

The lipid content per mosquito can be read directly from a calibration line. This line is obtained as follows. Use three sets of 50, 100, 200 and 400 μl of the standard solution (1 mg/ml), evaporate solvent and treat as above. Read optical density at 525 and 490 nm. The calibration should be repeated occasionally. The calibration line at 525 nm (Fig. 1) was obtained in a Coleman IIA spectrophotometer with linear absorbance.

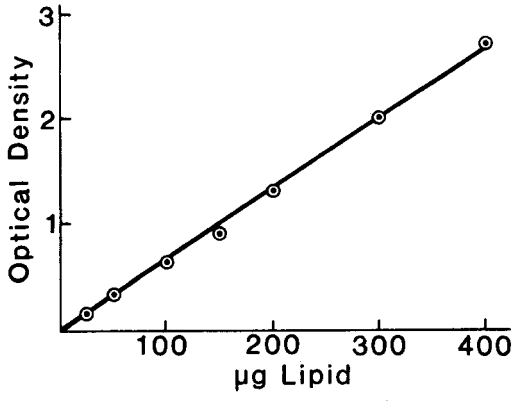

Fig. 1. Calibration line for soy bean oil at 525 nm. The values higher than 1 were obtained by dilution (see text).

DISCUSSION

In order to establish the validity of soy bean oil as a standard for mosquito lipids, female *Aedes aegypti* (Linn.), maintained on sucrose, were extracted with chloroform-methanol, and non-lipid material was removed by repeated extraction with water. The mosquito lipids were dried, and dissolved in chloroform (1 mg/ml). This solution gave optical densities identical with that of the soy bean oil solution (Fig. 1).

Using this procedure, the assay in the crude extract correlates closely with that in a more purified extract (Table 1). However, when the assay in the extracts was done with a non-specific reagent such as bichromate sulfuric acid (Van Handel 1984), the apparent lipid content in the crude extract exceeded that of the purified extract (results not shown). The variability of lipid content is largely due to variability of triglycerides. Starved mosquitoes held without food until they begin to die provide a baseline that include all non-nutritional and structural lipids. For most mosquito species this baseline varied between 20 and 35 μg. Values in populations that exceed this baseline are due entirely to metabolizable lipid reserves (Table 1). Gravid mosquitoes should be either excluded or the ovaries should be removed before the assay, since ovarian lipids have no established nutritional value for the female. The method is equally useful to determine total lipids in larvae and pupae. Separation of larval and pupal lipids of *Aedes aegypti* by thin layer chromatography established that over 90% consisted of triglycerides and phospholipids, the balance being free fatty acids, sterols and traces of hydrocarbons. The assay may be applicable to other small insects.

Table 1. Comparison of lipids in *Aedes aegypti* (μg/♀ ± S.E.) with and without purification of the crude extract.

	Purified extract	Crude extract
Starved to death	18 ± 3	18 ± 2
At adult emergence	40 ± 2	47 ± 3
Three days on 10% sucrose	190 ± 12	175 ± 10
Seven days on 10% sucrose	235 ± 20	215 ± 12

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