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BLOOD MEAL SIZE OF THE STABLE FLY, *STOMOXYS CALCITRANS*, MEASURED BY THE HiCN METHOD

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ABSTRACT. A chemical method for measuring the blood meal size of *Stomoxys calcitrans* was compared to gravimetric measurement. Results indicated that the hemoglobincyanide (HiCN) method gives reliable estimates of blood meal size and may be used under field conditions. This technique was used to measure blood meal sizes of 11.2 μl for males and 15.1 μl for females.

INTRODUCTION

Stable flies associated with livestock can cause severe injury, reduced milk production, and increased susceptibility of the weakened animals to death and disease (Bishopp 1913, Bruce and Decker 1958, Campbell et al. 1977). The economic losses attributed to stable flies on livestock have not been correlated with fly feeding activity, however. This is due largely to limitations of conventional techniques for measuring blood meal size in the field.

The commonly used gravimetric method is impractical under field conditions because the prefeeding weight of

flies attracted to a host is unknown. Blood meal size calculated by the difference between pre- and post-feeding weights may be in error by the amount of water and urine lost between weighings. Also, since host defensive behavior has been shown to affect the feeding success of mosquitoes (Edman and Kale 1971, Edman et al. 1974, Klowden and Lea 1978), controlled feeding experiments in which stable flies are allowed to feed to repletion (Anderson and Tempelis 1970) may not reflect the true blood meal size taken by the insects when the host is defensive.

Briegel et al. (1978) modified a standard clinical hemoglobinometric procedure for determining the blood volume ingested by mosquitoes. In this paper we report the use of their hemoglobin-cyanide (HiCN) method for measuring the blood meal size of *Stomoxys calcitrans* (L.).

MATERIALS AND METHODS

Larvae from a laboratory colony of *S. calcitrans* were reared in CSMA at 27° C. Our preliminary attempts to correlate gravimetric and HiCN blood meal determinations were unsatisfactory because flies would not feed for several hours after the pre-feeding anesthetization. As an alternative, we gave enemas of rat blood to previously weighed females and the blood volume determined gravimetrically (specific gravity of blood = 1.05) was related to the value obtained by the HiCN method. This method, which has been fully described by Briegel et al. (1978), involves the lysis of erythrocytes with Drabkin's reagent and the subsequent conversion of hemoglobin to hemoglobin-cyanide, a stable compound which can be evaluated spectrophotometrically. We removed the blood-filled midgut from each fly and placed it into 1 ml of Drabkin's reagent where it was dissociated with a motorized pestle and subsequently incubated at room temperature for 20 min. The optical density at 540 nm was determined with a spectrophotometer. A standard curve of host blood volume vs. optical density was used to relate the optical density of blood in the midgut to its volume.

We then allowed previously unfed, 2-day-old flies to feed to repletion on the anesthetized laboratory rat. The blood-filled midguts of these males and females were removed and treated as described above, and the blood volume estimated by relating the optical density of the sample to the standard curve.

The dry weights of unfed flies of each sex were determined by drying them at 45° C to constant weight.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between blood meal sizes calculated gravimetrically and by the HiCN method. The HiCN method provides results comparable to conventional gravimetric analysis. The replete blood volumes ingested by our male and female *S. calcitrans* measured by the HiCN method are shown in Table 1. The larger blood meal

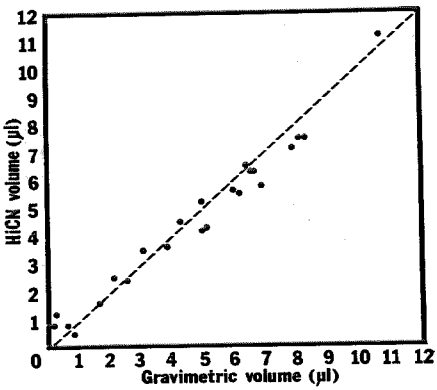

Fig. 1. Correlation between blood meal sizes calculated gravimetrically (X) and by the HiCN method (Y) for *Stomoxys calcitrans* (N = 23). The slope of the regression line (Y = 0.20 + 0.92X, R^2 = 0.97) does not differ significantly from 1 (t = 0.39); the Y-intercept does not differ significantly from 0 (t = 0.29).

Table 1. Weight and blood meal size of male and female *Stomoxys calcitrans* measured by the HiCN technique. Values are means and 1 standard deviation.

Sex	N	Weight (mg dry)	Blood Meal Size (μl)
Male	23	2.7 ± 0.3*	11.2 ± 2.4**
Female	23	3.3 ± 0.4	15.1 ± 2.8

* t test between sexes: $t = 5.0$, $P < 0.01$.
** $t = 7.1$, $P < 0.01$.

sizes ingested by females may be related to their greater dry weights.

Although we restricted our experiments with the stable fly to the laboratory, Klowden and Lea (1978) used the HiCN method to measure the amount of blood ingested by field populations of mosquitoes attracted to a stable trap containing a specific host. A sample of blood from this host was required for calibration. The HiCN technique can also be applied to *S. calcitrans* collected around a host within a stable trap or other enclosure.

Since the stable fly does not excrete hemoglobin for several hours after feeding (Bishopp 1913), the HiCN method does not require immediate analysis of blood meal size. The gravimetric method, however, requires immediate analysis and may underestimate the size of the blood meal by the amount of fluid lost between weighings. Limitations of the HiCN method are that flies must feed on a specific host from which a sample of blood must be taken for calibration, and that flies cannot be used for further experimentation following measurement of their blood meal sizes.

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