tend to limit the amount of droplet size testing which can be done.

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SIMPLE BIOLOGICAL AND CHEMICAL METHODS TO DETERMINE THE CALORIC RESERVES OF MOSQUITOES

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ABSTRACT. Two methods are described to determine the caloric contents of mosquitoes. The biological method depends on accumulative mortality in a non-fed population. The chemical method depends on complete oxidation of the mosquito with bichromate and spectrophotometric assay of reduced chromium.

The ability of a mosquito to survive, and therefore, to transmit disease depends largely on its caloric reserves. The two endogenous sources of fuel, fat and glycogen, disappear during starvation, and reappear after feeding on sugar or blood (Van Handel, 1965). In addition, the nature of the fuel used during sustained tethered flight has been determined (Nayar and Van Handel, 1971). The determination of fat and glycogen requires considerable effort and the usual "fat" and "glycogen" measurements include a fair amount of structural material that is not available as a caloric source. Moreover, when a mosquito has taken a nectar meal shortly before capture and is then maintained without further food, its glycogen and fat reserves will initially increase instead of decrease. Thus, these determinations do not necessarily show the total caloric energy available for activity. Two methods which do not show this defect have been developed.

SURVIVAL TIME WITHOUT FOOD. When mosquitoes are held at constant temperature in a clean plastic cage with only water available in a near-saturated atmosphere, the 50 percent mortality time is an accurate measure for biologically available reserves (Fig. 1), irrespective of the nature of these reserves. The biological method, however, requires fairly large numbers (50–100 mosquitoes per determination) and considerable surveillance, because dead mosquitoes have to be counted at frequent intervals in order to accurately establish the 50 percent mortality time. I have therefore made an attempt to supplement the biological method with a chemical method, requiring only a single reagent and minimal manipulation.

DETERMINATION OF BICHROMATE VALUES. An acid solution of bichromate oxidizes the entire mosquito, including protein, lipids, carbohydrates and chitin to CO_2, while reducing the red Cr^{VI} to the green Cr^{III} ion.

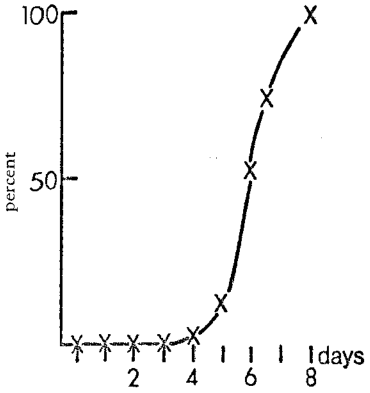

FIG. 1.—Cumulative mortality of *Aedes sollicitans* maintained from emergence at 25° C on water.

> Mix 20 gram sodium bichromate with 1 liter concentrated sulfuric acid at about 70° C. (After standing a few days at room temperature, this reagent should be decanted or filtered through glass wool.) Place individual mosquitoes in 16 x 100 ml tubes. Use at least one tube without a mosquito. Add 1 ml reagent. Heat at 100° C for 15 min, cool and slowly dilute with 1.5 ml water, while the tubes remain immersed in a water bath. Transfer to appropriate colorimeter tubes and read the optical density in a spectrophotometer at 605 nm against the reagent blank.

The color produced by 1 mg sugar (e.g. 0.1 ml 1 percent sucrose solution) can be used as a standard and is equivalent to 4 calories. If after a few minutes heating there appears to be too much material (this is the case when the color turns light green and undigested particles float in the reagent), an additional ml of reagent should be added and after heating for 15 min, 3 ml instead of 1.5 ml water. The optical density should then be multiplied by a factor of 2. It is practical to heat the 16 x 100 mm tubes in aluminum blocks provided with 17 x 50 mm holes, mounted on a hot plate (Thermolyne type 2200), provided with a thermometer placed in a tube filled with glycerol. Heating for more than 15 min or at temperatures up to 115 ° C does not affect the results.

In order to assess the relationship between caloric reserves and survival time without food, *Aedes sollicitans* were reared from eggs on a standard diet (Lea, 1963). Adult females were collected and maintained at 25 ° C with 12 hr. light, with only water available, in groups of 50, in plastic cups (400 ml). Another group was maintained on a 10 percent sucrose solution. Three cups were used per experiment to determine mortality (Fig. 1) and caloric reserves during starvation and feeding (Fig. 2).

Optical density (O.D.) was measured in a Coleman Junior Spectrophotometer in 12 mm round cuvettes. The O.D. produced by 1 mg sucrose was 0.38 and 1 cal was therefore equivalent to an O.D. of 0.095. The caloric reserves were calculated from the difference in bichromate value between the sample and the mosquitoes starved to death. The latter represent the irreducible minimum values. Each point represents the average of 10 mosquitoes determined individually (Fig. 2). The standard error was in no case higher than 5 percent of the mean. After

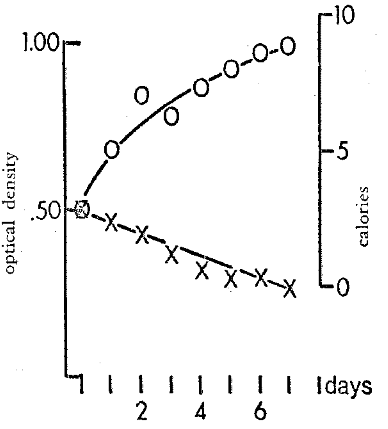

FIG. 2.—Bichromate values of *Aedes sollicitans*, starving (-x-x-) or feeding (-o-o-) from day of emergence.

TABLE 1.—Distribution of available calories in a field population of 100 female *Aedes taeniorhynchus.*

O.D. readings	0.10–0.20	0.21–0.30	0.31–0.40	0.41–0.50	0.51–0.60	0.61–0.70
Calories *	0–1	1–2	2–3	3–4	4–5	5–6
Percent of total	17	25	30	20	7	1

* The optical density of 0.15 has been used for the irreducible minimum value.

7 days of starvation, the O.D. was 0.28 ± 0.01 in surviving and 0.26 ± 0.006 in dead *A. sollicitans.* At that time, mortality was 87 percent, and the 50 percent mortality level was passed on the sixth day (Fig. 1).

This method may have merit for measuring the caloric reserves of field populations. It would not matter whether the reserves are present as nectar, blood, fat or glycogen. Of course, gravid females should be excepted because of the "non-caloric" bichromate value associated with oviposition. This is a minor problem, since light traps and baits are known to take a very small percentage of gravid females.

The distribution of bichromate values in a field population of *Aedes taeniorhynchus* is presented in Table 1. The mosquitoes were collected in suction traps and were frozen within 2 hours after capture. In order to determine the irreducible minimum value it is necessary to starve a group of the population to death.

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