QUANTITATIVE DETERMINATION OF FEEDING RATES OF *ANOPHELES ALBIMANUS* LARVAE [1]

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Much of the food of mosquito larvae consists of particulate matter which they extract from the water by a filter-feeding mechanism. Dissections of a number of species have revealed a wide variety of microorganisms and high proportions of both organic and inorganic detritus in the gut with little evidence of selectivity (Hinman, 1930; Pucat, 1965). Mosquito larvae apparently ingest all available particulate materials which can be swallowed without regard for their food value. The feeding mechanism does not function with equal efficiency on all kinds of particulates, however. Factors such as size, concentration, and chemical make-up of particulates may be expected to influence the feeding process. Dadd (1968) determined by direct observation of materials in the gut that *Culex pipiens* larvae ingest different inorganic particulates at varying rates and that materials of "broadly nutritive value" are more rapidly consumed than non-nutritive substances. For anophelines, which feed primarily at the water surface, the flotation and dispersal characteristics of a food material also have great significance. The rate at which they clear a given surface area of particles dusted on it has been used as a measure of ingestion for *Anopheles* spp. larvae (Clements, 1963). Such procedures yield comparative information about the rates at which various materials are consumed but do not indicate the absolute amounts ingested. Moreover, when frequent or continuous direct observations are required, the number of larvae which can be tested is limited. This paper outlines a colorimetric technique which permits simultaneous study of large numbers of larvae and allows quantitative expression of ingestion rates. The method, which is applicable to both surface-feeding and browsing larvae, has been applied in a study of particle size to feeding rate relationships with *Anopheles albimanus* larvae.

METHOD. Criteria for a test food material included acceptability to the larvae, affinity for a selected dye, flotation and dispersal qualities, and availability in a range of sizes. A summary of materials tried but found unsuitable because they failed to meet one or more of these criteria is given in Table 1. Whole wheat (graham) flour, a component of our standard insectary diet for anophelines, was selected as the test material. It has dyeing, flotation and dispersal qualities that are adequate for test purposes, and when passed through standard sieves, it yields a series of particle size ranges. The flour was sieved to the desired size range and dyed in a round bottom flask with water-insoluble Sudan Black-B in acetone; 25 mg. of dye were used per gram of flour. The acetone was removed with a rotary vacuum evaporator, and the dyed flour was resieved to assure uniformity. Its acceptability to the larvae had been established by direct observation with a binocular microscope. Ten isolated third-stage *A. albimanus* larvae, each continuously observed for 4 to 5 minutes, spent an average of 56.6 percent of the time feeding on undyed flour of 88–105μ size. The range was 19.5 percent to 98.5 percent. Ten additional third-stage larvae, when offered dyed flour of the same size, fed an average of 72.8 percent of the time, with a range of 24.0 percent to 100 percent.

Lots of 50 larvae selected for uniformity of size and instar were placed in fine-mesh wire baskets partly submerged in 2 cm. of tap water in individual 600-ml.

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TABLE 1.—Particulates tried as test food for anopheline feeding study

Material	Reasons for Rejection
Pollens	Size range inadequate
Ground corn meal	Particles failed to disperse
Charcoal	Particles too large
Norit A (decolorizing carbon)	Particles too small, coated larvae
Powdered cellulose	Too fibrous, clumped
Chromasorb 101 (styrene polymer)	Floated too high, did not wet
Diatomaceous earths:	
Chromasorb g, Q, W	Floated too high, did not wet
Diataport	Particles sank

beakers. The baskets provided 25 sq. cm. of water surface. Five mg. of the dyed food were sprinkled on the water inside each basket. Since larval activity tends to sink the food, 5 mg. increments were added at 10-minute intervals to insure an adequate supply of floating food throughout the test. After a predetermined feeding time the larvae in each basket were vigorously tumbled in a stream of tap water to remove clinging food particles. They were then removed from the basket, folded in a strip of filter paper, crushed, and air dried. The paper strips constitute individual records of the food ingested by the larvae in each lot of 50 and may be stored indefinitely at this point.

The dye was extracted from the filter paper and larvae with a known amount of acetone, and the resulting blue solution was read in a Beckman model B spectrophotometer [2] at 605 mμ, the peak absorbance wavelength of Sudan Black-B. The optical density of the unknown was compared with that of a color standard prepared from known weights of the same batch of dyed food. The ratio of concentration (C, mg. of food per ml. of solution) to optical density (D) was found in separate determinations over a range of concentrations to be a constant (K). It can, therefore, be expressed as K=C/D, and a single concentration is sufficient to determine it. In practice, however, three weighings each of approximately 10 and 20 mg. were extracted with 50 ml. of acetone, and K was estimated from mean values for concentration and optical density. By multiplying the optical density of any unknown by the K value derived from the same food sample, the concentration of the unknown (mg./ml.) may be determined and then expressed as μg. of food per larva.

RESULTS AND DISCUSSION. The use of sieve-opening dimensions to designate food size ranges is convenient but should not be considered highly accurate. The food particles were irregular in shape and, since the sieve openings were square, the length of the diagonal would be the limiting dimension for any plate-like particles. A square 105μ opening, for example, has a diagonal of 148.5μ, making possible the passage of flat particles with two dimensions of that magnitude. Discrete size categories were obtained by the use of sieves, however, and differences in rates of ingestion related to this parameter were demonstrated.

Figure 1 illustrates the application of the procedure to determination of the time-ingestion relationship for third-stage *A. albimanus* larvae feeding on 88–105μ food; Table 2 compares the ingestion of this food with that of four other size ranges. Samples of 50 larvae were assigned in pairs, one to each of two food sizes (*e.g.*, 88–105μ and 62–74μ) and allowed access to the food for 10, 20, 30, 40 or 50 minutes. These comparisons were made four times for each of the 20 food size pairs and access time combinations shown. The primary entries in Table 2 are the mean weights (micrograms) of food ingested per larva averaged over the four replicates.

Although ingestion of foods of all sizes increased with time, the 88–105μ food was always consumed in greater amounts than either smaller or larger sizes. Differences of at least 6.6 micrograms be-

[2] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

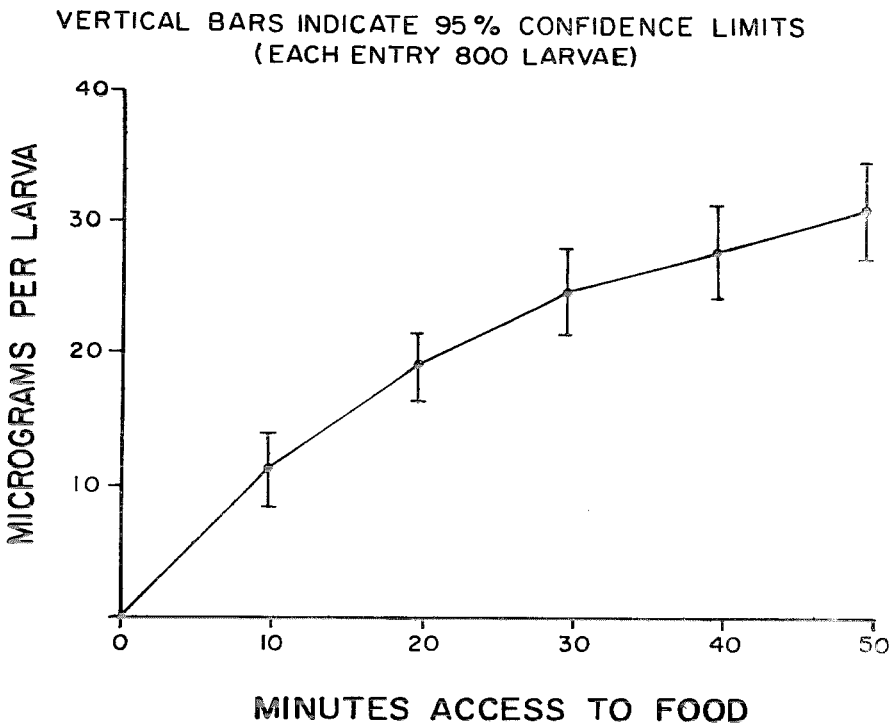

FIG. 1.—Mean ingestion of 88–105μ whole wheat flour by third-stage *A. albimanus* larvae.

tween the mean ingestion of two food sizes were statistically significant. Differences of this magnitude or more were obtained at each feeding interval in the comparisons of 88–105μ food with the 149–177μ and 177–250μ size ranges.

Jones (1960) noted that both ingestion and egestion rates were highly variable for *Anopheles quadrimaculatus* larvae of uniform appearance reared under controlled conditions. Similar variability characterized *A. albimanus* in this study. The differing mean values obtained for ingestion of the 88–105μ food (Table 2) can be attributed in part to variation in larval size, to their pretest nutritional history, and to the proportion of a sample in a pre-moult condition. The presence of a feeding stimulus is apparently an important factor also. In exploratory work with ragweed pollen, third-stage *A. albimanus* larvae, starved overnight in clean tap water, ingested the pollen at an average rate of 1.8 μg. per larva per hour. The ingestion rate of larvae fed overnight was nearly doubled (3.2 μg. per larva per hour).

An explanation for this apparent anomaly is suggested by the recent work of Dadd (1970a, b) who reported that yeast extract has a phagostimulant effect on *C. pipiens* larvae, which results in more filtering activity and faster consumption of several non-nutritive materials. The formulation used to rear *A. albimanus* larvae for the present study contains 25

TABLE 2.—Micrograms of food per larva ingested by third-stage *A. albimanus*. Parentheses show "t" values in tests of paired replicates.

Food Size (microns)	Minutes Access to Food				
	10	20	30	40	50
88–105	6.9	14.9	20.2	22.1	25.7
vs.					
62–74	6.1	11.7	15.2	16.9	20.6
Difference	0.8	3.2	5.0	5.2	5.1
	(1.11)	(2.53)	(1.73)	(2.01)	(1.86)
88–105	15.7	21.6	26.4	31.3	30.7
vs.					
125–149	10.7	19.9	25.0	26.7	28.5
Difference	5.0	1.7	1.4	4.6	2.2
	(2.12)	(1.59)	(1.30)	(1.32)	(1.04)
88–105	13.3	22.1	26.0	31.6	35.4
vs.					
149–177	6.7	15.3	19.3	20.5	25.0
Difference	6.6	6.8	6.7	11.1	10.4
	(17.17)**	(7.94)**	(17.92)**	(7.50)**	(5.59)*
88–105	9.4	17.9	26.5	26.5	32.5
vs.					
177–250	4.2	10.6	14.2	15.9	19.9
Difference	5.2	7.3	12.3	10.6	12.6
	(2.26)	(3.72)*	(10.62)**	(5.96)**	(6.75)**

** Difference significant at 1% level ($P_{0.01} = 5.84$).
* Difference significant at 5% level ($P_{0.05} = 3.18$).

percent brewer's yeast. The reduced pollen ingestion observed after starvation of these larvae by removal to clean tap water suggests that yeast extract may stimulate feeding by this species also.

Metcalf and Hess (1944) fed ground sand with diameters ranging from 1 to 300μ to *A. quadrimaculatus* larvae and found the largest size ingested by the third stage to be 99μ. Third stage *A. albimanus* larvae in the present study ingested considerably larger particles. Although differences in mouthpart dimensions between the two species may account in part for this discrepancy, the physical characteristics of the two particulates are more important. Observations with a binocular microscope of larvae feeding on wheat flour showed that these particles were sometimes elongated in their passage between the mandibles. The ability of the larvae to distort soft food materials allows ingestion of larger sizes than would be possible with hard, unyielding materials such as sand.

SUMMARY. A colorimetric technique using dyed food particles floated on the water has been developed for investigating the relation of particle size to food consumption by anopheline larvae. Simultaneous study of large numbers of larvae is possible with results expressed as μg. of food/larva/unit time. Tested with larvae of *A. albimanus*, the method has demonstrated the relation of food particle size to feeding efficiency.

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MULTIPLICATION OF CALIFORNIA ENCEPHALITIS VIRUS IN YUKON MOSQUITOES [1]

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Arising from the isolation of the Grey Sage strain of California encephalitis (CE) virus from a pool of *Aedes canadensis* and *A. vexans* mosquitoes collected near Penticton, B.C. (49° 30′N, 120°W) during June 1969 (McLean, 1970) plus detection of CE neutralizing antibody in sera of marmots (*Marmota flaviventris*) at Penticton (McLean *et al.,* 1970) and snowshoe hares (*Lepus americanus*) from Penticton north to Dawson Creek (56°N, 120°W) (McLean *et al.,* 1971, Newhouse *et al.,* 1963), attempts were undertaken during summer 1971 to define the northern limit of prevalence of CE virus in British Columbia and immediately northward in the Yukon Territory. Isolation of CE virus strains from *Aedes communis* and *A. stimulans* mosquitoes collected near Rochester, Alberta (54°N, 113°W) from 1965 through 1968 (Hoff *et al.,* 1969), and from *Aedes* spp. mosquitoes collected in east central Alaska (approximately 63° N, 145°W) during 1970 (Feltz *et al.,* 1971) further suggested the possibility of endemic prevalence of CE virus in the Yukon. Isolation of 12 strains of CE virus, antigenically identical with the Grey Sage strain by mouse neutralization tests, from *Aedes canadensis* collected near Whitehorse (61°N, 135°W) during June and July 1971 (McLean *et al.,* 1972) together with the demonstration of CE neutralizing antibody in sera from 76 of 298 (25 percent) *Lepus americanus* confirmed the existence of a natural cycle of infection of CE virus in the Yukon. Both the Grey Sage strain, and isolates from Alaska and Alberta show close antigenic relationships to the Montana snowshoe hare strain of C virus.

Aedes vexans and *A. canadensis* collected wild in Ontario, and also domes-

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