A METHOD FOR COMPARING FEEDING RATES IN MOSQUITO LARVAE

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When nutritional requirements are studied by comparisons of growth on variously modified defined diets it is important to know if rates of feeding are uniform, for unless they are, poor growth resulting from deficiencies in specific nutrients cannot readily be distinguished from effects that might be due to underfeeding (Dadd, 1960). If cursory observation can show that feeding is more or less normal, as with large insects ingesting solid material or producing prominent feces, precise comparisons of feeding rate may be less important, at least for qualitative study of major nutrient requirements. But if food ingestion is cryptic, and the feces not readily observable, the assumption of normal feeding is largely a matter of faith unless methods for monitoring ingestion rates are available. This is particularly the case with aquatic insects such as mosquito larvae that live in their food medium.

Mosquito larvae typically are filter-feeders, subsisting on microorganisms and particulate organic debris, supposedly with little ingestion of liquid (Christophers, 1960; Clements, 1963; Pucat, 1965). Ingestion of dissolved nutrients must be possible, however, since synthetic diets on which *Aedes aegypti* has completed development contained many of the essential nutrients in solution. Nevertheless, even with all nutrients present as solutes, such diets usually incorporated some insoluble material such as cellulose powder or agar (Singh and Brown, 1957; Lea, Dimond, and DeLong, 1956), and it appears that without solids, feeding is impaired and growth reduced (Nayar, 1967). It therefore seemed reasonable to monitor the rate of ingestion of the particulate phase of media for use in nutritional studies as an index of comparative rates of ingestion of nutrients as a whole.

Rates of ingestion of natural foods by mosquito larvae have been studied by observing the passage through the gut of identifiable particles such as carmine, or carbon particles, introduced into the culture medium (Christophers, 1960; Pucat, 1965). The method described below, a modification of this approach, involves filling the gut with a readily identifiable inert particulate, and then comparing the rates at which this is displaced on transfer of the larvae to media incorporating experimental particulates or diets.

On transfer from culture to chinese stick ink rubbed up in water to give a dense black suspension, essentially of fine carbon particles, larvae of *Culex pipiens* soon fill the peritrophic membrane within their midgut with carbon. This occurs in all instars, and since the larvae are transparent, the whole gut from esophagus to ileum then appears as a sharply defined black column. When left overnight in chinese ink, effectively all larvae thus glut themselves with carbon; the occasional exceptions were found to be injured or approaching pupation.

On transfer to clear water, larvae continue to make filtering movements of the mouthparts even though no filterable material is present. If previously glutted with ink, black fecal pellets may be expelled from time to time, but the column of carbon as a whole remains *in situ* from end to end of the gut. Apparently the accession of solid material anteriorly is necessary to move the column rearwards.

Displacement of the carbon column occurs as soon as the larvae are transferred to water containing a particulate such as yeast powder or diatomaceous earth. The new particulate, if light colored, may then be observed moving rearwards, while black fecal pellets are expelled at a greatly increased rate. The boundary between the carbon originally filling the gut and

the new particulate remains stable, so evidently no mixing or churning of the contents of the food column occurs. Timing of the rearwards movement of the boundary, and counts of the numbers of fecal pellets expelled, provide criteria for assessing comparative rates of ingestion of particulate dietary materials.

In initial experiments batches of 4th instar larvae were glutted with carbon overnight. Individuals were then transferred singly to small plastic dishes (the caps of specimen tubes) containing water with a layer of a suitable particulate covering the bottom of the dish. The dishes, 15 mm in diameter, were readily observed with little disturbance under the lowest power of the stereobinocular microscope. With a depth of water of about 6 mm, larvae could brush sedimented particulates with their mouthparts while suspended from the surface by their respiratory siphons. Observations were made about every 5 minutes as the boundary moved down the gut, until displacement of carbon was complete and the final black pellet expelled.

Two substances, dried yeast powder (dead) and the alga *Chlorella* (live), both of broadly nutritive value, consistently and rapidly displaced all carbon, in times ranging from 25 to 90 minutes, mostly in less than an hour. These times are of the order observed by previous workers for the passage through the gut of identifiable particles put in natural food media. Of a few inert particulates tested, kaolin and brick dust (pounded red house brick) sometimes displaced the carbon completely within an hour, but generally longer periods of up to 4 hours were needed. Complete displacement with chromatographic cellulose or diatomaceous earth required at least 90 minutes and in most cases was incomplete when observation was terminated after 4 hours.

These findings showed that different particulate materials varied widely in the rate at which larvae ingested them, but a drawback to the use of total displacement times as a routine measure of comparative ingestion rates is that observations must be made continually and at frequent intervals, in many cases for several hours. It was therefore more convenient to allow the larvae a set access time for all treatments, and then to record the extent of the displacement of the boundary between carbon and experimental particulate. At the same time, a count of the number of black fecal pellets that were expelled provided an alternative criterion of rate of ingestion.

Displacement of the boundary was more readily recorded in morphological terms than by actual measurement. Apart from convenience, variability in the data due to the natural variability in larval length was thereby obviated. Larvae were assigned displacement values according to the system illustrated in the diagrams of Fig. 1. If the boundary had reached the posterior margin of the thorax, a value of 1 was assigned. Values of 1½, 2, 2½, 3, —6 were given when the boundary was at the mid-point or posterior margin of the 1st to 5th abdominal segments respectively. The midgut terminates between segments 5 and 6, beyond which the food column, up to this point an apparently uniform cylinder, is formed into fecal pellets in the hindgut. A value of 6½ was assigned if carbon was completely displaced from the midgut but still remained as pellets in the hindgut, and a value of 7 when no carbon remained in the larvae. Values of 0 and ½ were respectively assigned if no displacement had occurred, or if the experimental particulate had advanced only half way through the thorax. In old 4th instar larvae, especially those approaching pupation, the thorax becomes opaque, when these latter two values can be distinguished only after dissection.

Generally the access period allowed was 1 hour. The majority of larvae given dried yeast (the most rapidly ingested material so far tested) had by this time completely displaced the carbon, and mean displacement values approximated to the maximum possible, 7.0, while pellet counts were likewise maximal, at about 35.

As an example of the method, table 1 presents data obtained in two experiments

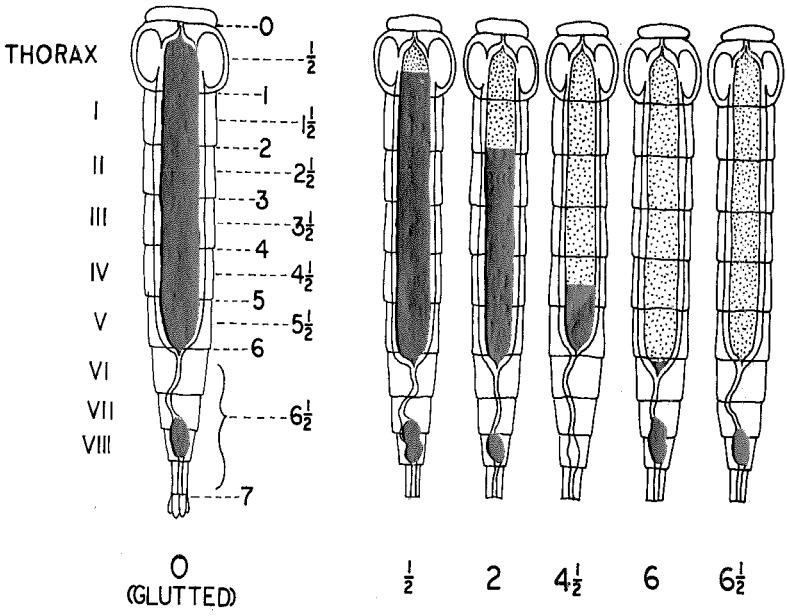

FIG. 1.—Diagrams of particulate-filled peritrophic membranes within the gut of mosquito larvae. The labelled diagram represents a glutted larva with chinese ink completely filling the peritrophic membrane. Roman numerals denote abdominal segments. Arabic numerals to the left give the displacement values that would be assigned if the boundary between ink (black) and an experimental particulate (stippled) reached the positions indicated. Unlabelled diagrams illustrate various positions of a boundary with appropriate displacement values given below.

in which ingestion rates were compared on dried yeast, *Chlorella,* brick dust, kaolin, and chromatographic cellulose powder. To prepare the experiments, small dishes were set up as before with water and the appropriate particulates. For *Chlorella,* a culture of the alga was shaken and the dispersed algae in the culture medium used without adding water. To start the experiment, larvae that had been glutted with carbon the previous day and held overnight in water were strained off and transferred, one to an experimental dish, by means of a fine brush. The time was noted and the dishes set aside for an hour, at the termination of which, displacement values and pellet counts were recorded for each larva. In these small pilot experiments, recording was carried out without immobilisation of the larvae, taking about 5–10 minutes. With larger experiments, to equalise access times it is necessary to immobilise all larvae by placing them in a freezer at 0° C. until recording.

The results shown in table 1 further demonstrate how variable the rates of ingestion of different particulates may be. Pellet counts follow much the same pattern as the more direct indication of ingestion rate given by displacement values, but they are evidently a more variable and less sensitive measure. Considerable numbers of pellets were sometimes expelled when displacement values showed that little or no ingestion had occurred. Furthermore, in other experiments, pellets have sometimes been difficult or impossible to count accurately. This was particularly so if an experiment was carried out shortly after removing glutted larvae from chinese ink and without an overnight period in water, for pellets then tended to crumble as soon as expelled.

The only difficulty encountered in re-

TABLE 1.—Displacement values and pellet counts for carbon glutted larvae allowed an access time of 1 hour with various particulates in water.

	Displacement values					Pellet counts				
	Dried Yeast	Chlorella	Kaolin	Brick Dust	Chrom. Cellulose	Dried Yeast	Chlorella	Kaolin	Brick Dust	Chrom. Cellulose
Exp. A	5	7	3½	2	½	38	26	22	21	16
	6½	6½	3½	3½	½	42	38	21	27	6
	7	7	7	2	½	21	37	34	15	15
	6½	3½	4	2	2	39	20	25	12	14
	7	3½	6½	3½	2	36	20	37	28	20
Exp. B	7	7	6	3	0	35	38	30	19	5
	7	7	5	2	0	27	32	27	18	4
	5½	7	4½	0	2½	22	30	19	5	16
	7	injured	0	1½	½	33	injured	9	11	5
	7	7	4½	2½	3	32	25	24	15	18
Means:	6.6	6.2	4.4	2.2	1.2	33	29	25	17	12

cording displacement values stemmed from the crumbling of pellets. If this happened, the carbon re-dispersed and could be re-ingested by the larvae, so that the gut again appeared black. Fortunately the original boundary between glutted carbon and experimental particulate was always apparent, presumably because no fecal material was present to be ingested right at the start of the access period. Moreover, the color due to reingestion of carbon was less black than that of the original glutted carbon, since fecal carbon was always admixed with the experimental particulate. In doubtful cases this was easily seen after closer examination of the dissected midgut.

With actively filtering larvae it may be anticipated that ingestion rate would be governed partly by such physical characteristics as particle size, material density, and concentration of particles. The consistently rapid ingestion observed with nutritive materials suggests that chemical factors may provide stimuli affecting feeding rates. Current work indicates that both physical and chemical factors are involved in regulating the rates at which particulate material is ingested. Results of this work will be presented in a subsequent paper.

SUMMARY. A method for comparing the rates of ingestion of particulate materials by mosquito larvae is described. Larvae are placed in chinese ink and allowed to completely fill their guts with carbon particles. They are then transferred for a set access period to water containing experimental particulates, and the rate of ingestion is compared in terms of the extent of displacement of carbon by the ingested experimental particulate. A displacement value, defined in morphological terms, is assigned by recording the position to which the boundary between carbon and experimental particulate has moved. A count of the black fecal pellets expelled during the access period provides an alternative criterion of ingestion. Diverse particulates were found to be ingested at widely differing rates, most rapidly in the case of materials of broadly nutritive value.

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