CHANGES IN WEIGHT AND CONCENTRATION OF SOLUTIONS OF DEXTROSE IN THE CROPS OF MOSQUITOES[1]

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ABSTRACT. *Aedes triseriatus* were fed solutions of dextrose in water, and the permeability of the crop to water and sugar was considered both outside the mosquito and *in vivo*. *Eretmapodites chrysogaster*, *Anopheles quadrimaculatus*, and *Aedes aegypti* were also used in certain experiments. The crops of sugar-fed mosquitoes, when removed from the mosquitoes and exposed to air, wrinkle within an hour or two indicating loss of content. Weight studies showed a significant loss of weight. Further studies involving both weight loss and changes in concentration of a contained sugar solution, showed that the weight loss is due to loss of water. Crops were removed from sugar-fed mosquitoes and held in distilled water for 24 hours. No changes in concentration were noted in the contained sugar solutions even though the crops were immersed in a hypotonic solution. These tests suggest that the crop is highly impermeable when kept wet. In live mosquitoes the crop content is reduced in amount as the sugar solution is slowly transferred to the midgut, but no significant concentration change was noted in samples taken from crops up to 12 hours after feeding. Thus, while in the live mosquito, the crop is highly impermeable to water and to dextrose.

Sugar solutions, mainly in the form of plant nectars, are important energy sources for both male and female mosquitoes. Ingested sugar solutions are directed into the three diverticula which arise from the oesophagus just anterior to the midgut region (Trembley, 1952 and Hosoi, 1954, 1959). Small amounts of solution are occasionally found in two small dorsal diverticula which lie between the flight muscles, but the bulk of the sugar meal enters a large ventral diverticulum, or crop, which lies in the abdomen.

Concerning the crop, Nuttall and Shipley (1903) wrote: "If a ventral sac is removed to a slide, and the salt solution or water in which it is suspended is allowed to dry, the sac does not collapse. The fluid it contains does not evaporate for a considerable time. Such sacs have been kept for several weeks in the laboratory, exposed to room temperature and unprotected, and still were found to contain fluid after as long as two months, by which time they had shrunk somewhat." This observation has been widely cited for many years as evidence that the crop is highly impermeable to water. In his book on the physiology of mosquitoes Clements (1963, page 134) states, "The walls of the diverticula are extremely impervious to water, for when a crop containing water was isolated and allowed to dry, it still contained water after 2 months (Nuttall and Shipley, 1903)." Denisova (1943) considered the water economy of mosquitoes and stated that the crop is impermeable to water but no supporting data were included.

It has been noted in our laboratory over a period of several years that diverticula containing sugar solution and exposed to the air, wrinkle and diminish considerably in size within a few hours. Their appearance is one resembling a loss of crop content. The difference between our observations and those of Nuttall and Shipley stimulated this study to determine if water is quickly lost from crops dissected from mosquitoes, and particularly to determine if the crop is permeable to water or to dextrose while in the living mosquito.

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MATERIALS AND PROCEDURES

The species used was primarily *Aedes triseriatus* (Say). Some comparisons have been made with *Aedes aegypti* (L.), *Eretmapodites chrysogaster* Graham, and *Anopheles quadrimaculatus* Say. Adult females were used.

The mosquitoes, from time of emergence, had access only to water and it was removed at least 3 hours before feeding sugar solution, so that the crop would be empty prior to feeding.

Dextrose was weighed and dissolved in double distilled, demineralized water. Concentrations of 10, 20, 50, and 70 percent were prepared in the proportions of dextrose in grams per 100 ml of solution. The mosquitoes fed quickly from cotton pads saturated with sugar solution; they were anesthetized with nitrogen prior to dissection in saline. Only the crop was studied because a dorsal diverticulum does not contain enough fluid to obtain a 0.2 μl sample.

Isolated crops, on which weight determinations were made, were transferred to small weighing pans of aluminum foil or plastic-coated paper discs and exposed to the atmosphere. Weighing was done with a Cahn Model M-10 electrobalance. The room temperature was approximately 75° to 80° F, and relative humidity was approximately 30 to 40 percent.

To determine the concentration of a sugar solution in a crop removed from a mosquito, a 0.2μl sample was withdrawn from the crop with a 0.5μl Hamilton Micro-Syringe, No. 7000.5-N. The sample was diluted 1:10,000 with distilled water and used for carbohydrate analysis (Morris, 1948). A 3 ml sample of fresh anthrone reagent (100 mg of anthrone in 50 ml of concentrated sulfuric acid) was placed in a test tube which was held in an ice bath. One ml of the diluted sugar solution sample was added and the content of the test tube was stirred well. A cap of aluminum foil was placed over the mouth of each test tube to retard evaporation, and all tubes were heated in a water bath at 100° C for 10 minutes, removed, and cooled in ice water. Optical density was determined at 620 mμ wavelength in a spectrophotometer and compared with a blank consisting of 1 ml of distilled water in 3 ml of anthrone reagent. Control samples were taken from the stock sugar solution used in each test.

The data were analyzed using the technique of analysis of variance and analysis of variance in regression (Ostle, 1954). The computations were performed on an IBM 7094.

RESULTS AND DISCUSSION

WEIGHT CHANGES IN THE CROP EXPOSED TO AIR. Weight studies were carried out to determine whether the contents of the exposed crop are lost as slowly as suggested in the literature. Crops were placed on small, individual weighing pans and weighed at intervals. Initial tests were carried out with six *E. chrysogaster* fed a 50 percent sugar solution. Each of the six crops lost weight during 1½ hours in air (Table 1). When six *A. triseriatus* were fed a 50 percent solution and their crops weighed for 50 hours, a weight loss also occurred. The data from these experiments are in Table 1.

Next, weight loss was measured in exposed crops containing a less concentrated solution of sugar. One mosquito of each of the species, *A. triseriatus, A. aegypti,* and *An. quadrimaculatus* was fed a 20 percent sugar solution. Weighings were made at intervals up to 73 hours. Each crop continued to lose weight until the 49th hour. In the order as given above, a weight loss of 1.190, 0.905, and 1.000 mg was noted for each specimen.

In the four species tested, the results indicated that a crop filled with a sugar solution loses considerable weight when exposed to the air for a few hours. No attempt was made to determine the rate of loss. This determination would involve the concentration and amount of the sugar solution, the temperature, relative humidity, and the surface area of each crop.

WEIGHT AND SUGAR CONCENTRATION

TABLE 1.—Weight changes of crops exposed to air. Crops were dissected from *E. chrysogaster* and *A. triseriatus* immediately after they were fed 50% dextrose.

	Weight of crop plus pan in mg			Analysis of variance in regression
	Initial	Final	Total loss	
E. chrysogaster (held 1½ hour)				
Crop 1	4.83	1.06	3.77	
2	2.42	2.10	0.32	
3	3.36	1.03	2.33	F_L 1.809*
4	2.51	0.63	1.88	F_B18.490**
5	3.54	2.19	1.35	
6	1.76	0.82	0.94	
A. triseriatus (held 50 hours)				
Crop 1	2.255	1.590	0.665	
2	2.950	2.025	0.925	
3	2.620	1.825	0.795	F_L 0.825*
4	2.835	1.900	0.935	F_B12.254**
5	1.775	1.355	0.420	
6	2.335	1.765	0.570	

* Not significant at 1% level.
** Significant at 1% level.

CHANGES IN CROPS EXPOSED TO AIR. To get some insight into what is lost during the period that a crop is exposed to air, evidence of concentration change in the contained sugar solution was sought. Weighings were taken of exposed crops at various intervals. At the termination of the weighings, a sample of the fluid inside each crop was taken, and its optical density determined. The optical density readings were then compared with control samples from the stock solution initially fed to the mosquitoes. An increase in concentration of sugar within a crop, would indicate a loss of water.

A. triseriatus were fed a 10 percent sugar solution. Immediately the crops were removed, placed on weighing pans, and held for 5 hours. In the six crops exposed to air, a loss of weight and an increase in concentration of the sugar solution occurred for each over the 5-hour period (Table 2).

A similar test was carried out with *A. triseriatus* fed a 20 percent solution. Four crops were exposed to air for 5 hours, and a weight loss and an increase in concentration occurred in each (Table 2).

A. triseriatus and *An. quadrimaculatus* were fed a 50 percent solution. Two groups of mosquitoes were used. The first group of four individuals, two of each species, was tested for concentration changes only. The second group, consisting of two *An. quadrimaculatus*, was tested for both weight loss and concentration changes. A significant increase in concentration took place in the crops of both species, and corresponding weight losses were noted for the exposed crops in the second group (Table 2).

The optical density readings of sugar solutions from the exposed crops showed an increase in sugar concentration indicating that water was lost. The walls of the mosquito crop consist of an inner noncellular cuticle, a thin epithelium, and an outer muscle layer (Clay and Venard, 1972). As a crop is exposed to the air, the outer network of muscle tissue and the epithelial layer desiccate and shrink. It would seem that the cuticle offers resistance to the passage of water, and that drying alters this layer directly or perhaps indirectly by the desiccated cellular tissues that surround it. Therefore, tests were carried out to determine the permeability of the crop wall when the crop was submerged in water.

SUGAR CONCENTRATION STUDIES IN THE

TABLE 2.—Changes in weight and the optical density of samples from crops which were exposed to air. The mosquitoes were fed 3 different concentrations of dextrose solution prior to dissection.

Species	Dextrose %	Hours exposed	Weight in mg Initial	Final	Total loss	Optical density	Analysis of variance
A. triseriatus	10	5	2.190**	2.030**	0.160	.165	
			2.200	1.500	0.700	.410	
			3.935	2.840	1.095	.335	
			2.145	1.805	0.340	.265	F =8.295*
			2.095	1.555	0.540	.350	
			3.565	3.195	0.370	.200	
						Control samples	
						.175	
						.135	
						.140	
						.140	
A. triseriatus	20	5	2.000	1.840	0.160	.365	
			2.390	1.595	0.795	.610	
			2.510	2.020	0.490	.570	F= 8.360*
			2.840	2.190	0.650	.430	
						Control samples	
						.310	
						.320	
						.320	
						.350	
A. triseriatus	50	1	.....	.....	.....	.640	
An. quadrimaculatus			.....	.....	.....	.640	
A. triseriatus		2	.....	.....	.....	.750	F= 7.282*
An. quadrimaculatus			.....	.....	.....	.900	
						Control samples	
						.670	
						.580	
						.600	
An. quadrimaculatus	50	1¼	2.170	2.035	0.135	.770	
			1.995	1.890	0.105	.760	F=17.537*
						Control samples	
						.580	
						.600	
						.670	

* Significant 5% level.
** All initial and final weights include weight of pan.

CROP HELD IN WATER. *A. triseriatus* were fed a 50 percent sugar solution, and the crops were immediately dissected free and held in distilled water for 24 hours before sampling. The optical density readings obtained from these samples were compared with control samples taken from the stock solutions initially fed to the mosquitoes. No significant concentration changes occurred (Table 3).

In two additional tests carried out with *A. triseriatus* and *An. quadrimaculatus* fed 50 percent solutions, similar results were obtained.

In the above tests, the contents of the crops did not change in concentration even though the organs were held in a hypotonic solution. Since the crop appears to be highly impermeable to water when kept wet *in vitro*, studies were made to determine if this were true *in vivo*.

SUGAR CONCENTRATION STUDIES IN THE CROP INSIDE THE LIVE MOSQUITO. Any gain in sugar concentration of the con-

TABLE 3.—Optical density of dextrose in crops held in distilled water for 24 hours compared with optical density of 50% stock solution originally fed to the mosquitoes.

Species	Optical density	Analysis of variance
A. triseriatus	0.625	
	0.690	
	0.650	
	Control samples	
	0.660	F=3.184*
	0.640	
	0.620	
	0.580	
	0.650	
	0.650	

* Not significant 5% level.

TABLE 4.—Optical density of samples removed from crops dissected from *Anopheles quadrimaculatus* at intervals after they were fed a 10% dextrose solution.

Hours after feeding	Optical density from each specimen sampled: Test 1	Test 2
0	0.150	0.152
	0.159	0.150
	0.152	0.150
3	0.142	0.151
	0.149	0.153
	0.161	
6	0.139	0.159
	0.155	0.150
		0.155
		0.151
Control samples	0.155	0.153
	0.142	0.155
		0.151
Analysis of variance	F=0.374*	F=0.7401*

* Not significant 5% level.

tained solution inside the crop would indicate a loss of water, while any decrease in concentration would indicate a gain of water, a loss of sugar, or dilution with saliva.

The first consideration was to determine any initial change in sugar concentration during or immediately after feeding. Seventeen *An. quadrimaculatus* were fed a 10 percent sugar solution, and two replicate tests were carried out. Three mosquitoes in each test were dissectd immediately after feeding, and samples of fluid from the crops were tested for optical density. No measurable concentration changes were observed, thus the sugar solution was not measurably diluted with saliva or with fluids already in the crops. Samples taken from the remaining 11 mosquitoes 3 or 6 hours after feeding likewise remained unchanged (Table 4).

Tests with *A. triseriatus* of different ages produced essentially the same results. Three mosquitoes, 4 days of age, and four mosquitoes, 7 days of age, were fed a 50 percent sugar solution. Samples of fluid were immediately removed from the crops and tested for optical density. The results showed that no significant change in concentration occurred during feeding.

To discover whether any concentration change occurred when sugar solution was retained in the crop for several hours, *A. triseriatus* were fed a 50 percent dextrose solution, and samples of fluid were taken from crops dissected at intervals thereafter. Replicate tests were conducted in some instances. No significant change in optical density was noted in the sugar solution within the crop immediately after feeding or at any time tested up to 12 hours after feeding (Table 5).

In a similar experiment, crop samples were taken from 24 *A. triseriatus* which had fed on a 70 percent dextrose solution. Two or more replicate samples taken from crops dissected at 1, 3, 6, 9, and 12 hour intervals, showed no significant change in concentration during the 12 hours after feeding. Despite a high concentration of sugar solution within the crop, there is apparently no movement of water from the haemolymph into the crop. This experiment and the preceding one could not be carried out beyond 12 hours because the transfer of sugar solution from the crop to the midgut did not leave a sufficient amount of solution in the crop for the anthrone test.

The results obtained for *A. triseriatus* and *An. quadrimaculatus* suggest that *in vivo* the crop wall is highly impermeable to water and to dextrose.

TABLE 5.—Optical density of samples removed from crops dissected from *Aedes triseriatus* at intervals after they were fed a 50% dextrose solution.

	Optical density from each specimen sampled		
Hours after feeding	Test 1	Test 2	Test 3
0	0.760 0.765	0.710 0.780 0.830	0.760 0.790 0.780 0.790
1½	.....	0.775 0.780	.....
2	0.790 0.725 0.755 0.945	.....	.....
3	.....	0.695 0.800 0.790 0.730	0.790 0.790 0.740
8	.....	.....	0.750 0.740
12	.....	.....	0.770 0.740 0.760
Control samples	0.720 0.760 0.715 0.845	0.750 0.730	0.780 0.770 0.790
Analysis of variance	F=0.391*	F=0.326	F=2.030*

* Not significant 5% level.

The dorsal and ventral diverticula in *A. triseriatus* are lined with a thin cuticle which is composed of 2 layers; a thin osmiophilic lipid layer lines the lumen, and adjacent to it is a less dense layer which is resistant to potassium hydroxide treatment (Clay and Venard, 1972). The lipid layer, or intima, appears as a thin black line in electron micrographs; apparently this lipid layer, which also lines the adjacent oesophagus, renders the walls impermeable. Hecker and Bleiker (1972), reporting on the fine structure of the ventral diverticulum in *A. aegypti,* also concluded that the cuticular lining of the crop is impermeable. The lateral diverticulum of the oesophagus, or the crop, in the blow fly, *Calliphora erythrocephala,* is lined with an epicuticle containing an electron-dense layer similar to the intima in mosquitoes (Smith, 1968).

Treherne (1957) studied glucose absorption in the cockroach, *Periplaneta americana.* He demonstrated quantitatively no uptake of sugar from the crop, and absorption was largely confined to the midgut caeca.

Contrary to the earlier statements made by Nuttall and Shipley (1903), the mosquito crop when exposed to air desiccates and shrinks; the exposed crop wall is in some way altered, and water is lost. If however, the crop is held in distilled water, and thus kept moist, the wall remains impermeable for at least 24 hours. Within the live mosquito, the crop wall is highly impermeable, and sugar solutions contained within the crop are slowly transferred to the midgut for absorption.

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A COMPARISON OF OIL AND FLIT MLO FOR CATCH BASIN TREATMENT[1]

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ABSTRACT. Flit MLO® performed satisfactorily in controlling *Culex pipiens* L. breeding in catch basins when applied at the rate of 1 ounce per basin. The use of Flit MLO was slightly more economical than the use of fuel oil.

INTRODUCTION. In some East Coast municipalities the design of older catch basins allows water to remain trapped, creating an ideal breeding situation for the common urban mosquito, *Culex pipiens* L.

Currently in New Jersey some control agencies employ either #2 fuel oil with or without emulsifier, Baytex, or Abate to control such mosquito populations. With an increasing concern for the environment, there has been a trend to limit their use particularly in those areas where contamination of lakes and streams may occur during run-off. While several agencies employ Flit MLO® (Exxon Company, U.S.A.) in other larval habitats the paucity of published information on its performance in catch basins warranted the following study. The purpose of this study, therefore, was to conduct a field evaluation of the effectiveness and economy of this and a related larvicide mixture which may be considered less polluting to the environment.

MATERIALS AND METHODS. Five blocks in Highland Park, New Jersey were set aside for the study. Catch basins in this area were excluded from the routine spray schedule conducted by the Middlesex County Mosquito Extermination Commission. Three larvicides, Flit MLO, #2 fuel oil without emulsifier and a mixture containing Triton X-207 (Rohm and Haas,

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