REGULATION OF THRESHOLD TO SUCROSE IN A MOSQUITO, *CULISETA INORNATA* (WILLISTON)

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Investigations on the contact chemoreceptors of various animal genera suggest that there are apparently sweet, salt and bitter taste modalities in insects and other invertebrates as well as in vertebrates. Thresholds of response to chemical stimuli not only vary in different animals, but also vary within the same species depending on the general physiological state of the animals. As early as 1922, Minnich (1922) showed that the labellar threshold response to sucrose solutions varied directly with the nutritional state of the butterfly, *Pyrameis atalanta*. However, this he did not find to be true of the oral lobes of the blowfly, *Phormia regina* (Minnich, 1922). Crow (1932) found that the tarsal threshold of response to sucrose in blowflies, *Lucilia sericata, Calliphora vomitoria, Cynomyia cadaverina* and *Calliphora erythrocephala* fell gradually as the period of inanition increased.

To date, no attempts have been made to determine the effect of nutritional state on the acceptance thresholds of sucrose in mosquitoes. This paper describes the influence of starvation and engorgement of sucrose and blood on the labellar thresholds of sucrose in a mosquito, *Culiseta inornata*.

MATERIALS AND METHODS. (General) The mosquitoes used in this study were obtained from a laboratory colony maintained in an air-conditioned insectary in the Department of Zoology and Physiology at the University of Wyoming.

The mosquitoes were anesthetized and attached to applicator sticks by the method described by Fier, Lengy and Owen (1961). After mounting, the front legs were removed at the trochanterfemoral junction to prevent grasping of objects; the specimens were allowed 16–24 hours to recover completely from anesthesia. Before testing, the mosquitoes were allowed to drink water to satiety. The acceptance thresholds were determined by presenting sucrose solution in ascending order to 3 or 4 labellar hairs; spreading of the labellum was used as an index of positive response. In each experiment fifty female mosquitoes were used. The data were analyzed by analysis of variance, one-way classification, and Duncan's new multiple range test.

All mosquitoes were kept on a diet of water only and each day were allowed to drink to satiety before testing. The tests began with mosquitoes 2 days of age and continued through the seventh day.

EFFECT OF FEEDING SUCROSE. Four-day mosquitoes, previously on a diet of water only, were used to determine labellar thresholds to sucrose. Immediately after the determination of thresholds, the insects were offered a 500.00 millimolar (mM) sucrose solution and were allowed to drink to repletion and the thresholds, were determined again. Immediately after this second determination of thresholds, the ingested sucrose solution was aspirated out of the ventral diverticula with an 80 gauge hypodermic needle. The thresholds were again measured after the aspiration of the sucrose solution.

In another experiment the thresholds were determined 10 minutes, 24, 48, 72 and 96 hours after ingestion of 500 mM sucrose solution.

EFFECT OF INGESTION OF BLOOD. After determining the initial thresholds of 4-day

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old hungry mosquitoes, they were fed defibrinated sheep blood which was mixed with sucrose solution to give a final concentration of 30.00 mM of sucrose. The mosquitoes readily imbibed the blood when it was presented to them on a cotton pad placed on a test tube filled with warm water (40–42° C). Immediately following the ingestion of blood in sucrose thresholds for sucrose were obtained. After each test the mosquitoes were dissected and the midguts were examined for the presence of blood. Only the results obtained with mosquitoes having almost all of the ingested blood in the midguts were used for calculation of mean acceptance threshold.

Results. For each successive day of starvation, the mean threshold values are shown in Table 1. There was significant difference between threshold values for mosquitoes starved for 2 days and those starved for 3, 4, 5, 6 and 7 days; but from the third day onward there was no statistical difference as the period of inanition progressed. During the course of this study it was observed that after the fifth day of starvation, the mosquitoes began to die in significant numbers. After the seventh day, the rate of mortality was as high as 95 percent.

Table 2 shows the effect of engorgement of 500.00 mM sucrose solution and blood on the labellar threshold of sucrose after feeding. The levels of threshold to sucrose increased about 16 times when the mosquitoes were fed sucrose but only 7 times when they were fed blood. When the ingested sucrose was aspirated, the level of threshold fell to the initial value. No attempt was made to aspirate the blood ingested by the mosquitoes. It is apparent from Table 3 that the threshold began to fall 24 hours after feeding sucrose and thereafter kept falling until the termination of the experiment. The threshold differences between each treatment were significant at 95 percent level with the exception of the difference between 48 and 72 hours.

Discussion. When mosquitoes were tested for the effect of starvation on the taste threshold, it was found that the threshold fell to the lowest level on the third day. Arab (1959) demonstrated that when blowflies were starved, a much lower concentration of sucrose was required to obtain a proboscis response than was required with a fed fly. He postulated that in the central nervous system there is a central excitatory state which can be excited or depressed by certain factors. Starvation, he found, raised the central excitatory state (CES); when the CES is high, a much lower concentration of sugar is needed to produce a response. The threshold to sucrose in mosquitoes starved from the day of emergence reached the lowest level within 24 hours, while in mosquitoes which were fed to repletion

Table 1.—Labellar thresholds for sucrose solutions of starved mosquitoes

Period of inanition (Days)	2	3	4	5	6	7
Mean thresholds (Millimolar)	109.75	44.51	32.31	30.99	29.58	29.58

Table 2. Changes in labellar thresholds* for sucrose following ingestion of 500 mM sucrose and blood

Solutions ingested	Mean threshold before feeding	Mean threshold after feeding	Mean threshold after aspiration
Sucrose	41.54	655.00	61.23
Blood + sucrose	37.32	257.50	..

* In millimolar concentrations.

TABLE 3.—Changes in labellar thresholds* for sucrose following ingestion of 500.00 mM sucrose solution

Mean threshold before feeding	Mean thresholds in hours after feeding				
	0.16	24	48	72	96
35.21	660.00	331.87	176.56	126.30	42.36

* In millimolar concentrations.

on sucrose solution the threshold fell gradually and it took 96 hours to reach the same level of threshold. The latter was also the case in the blowfly as shown by Evans and Dethier (1957). They reported that the acceptance threshold to sugars in the blowfly was elevated shortly after feeding and fell gradually to a low level in about 25 to 100 hours depending on the kind of sugar ingested by the flies.

Later it was demonstrated by Dethier and Bodenstein (1958) and Gelperin (1966) that duration of threshold elevation in the blowfly depends on the duration of crop emptying. They postulated that in the foregut of the blowfly there is a stretch receptor which senses the pattern of foregut distention as the ingested solutions move from the crop to the midgut. The information from this receptor is relayed via the recurrent nerve to the brain, where it inhibits the input from external receptors, resulting in increased threshold. With the passage of time, the crop becomes empty, the distention pattern of the foregut changes, and the sensory input from the external receptors is inhibited to a lesser degree. This is observed behaviorally as a decreased threshold.

The gradual decrease in the labellar threshold of *C. inornata* to sucrose following ingestion of sucrose, suggests that perhaps this mechanism is also operating in this species of mosquito. Besides the foregut receptor, if it is present in *C. inornata*, presence or absence of pressure in the abdominal cavity may also be responsible for the regulation of taste threshold. The evidence for this suggestion comes from the facts that when the mosquito was fed on sucrose solution, the labellar threshold was elevated; but was returned to the initial value soon after aspiration of the sucrose solution from the ventral diverticulum; the threshold also increased immediately following the ingestion of blood which entered primarily the midgut. Dethier and Gelperin (1967) suggested that feeding in the blowfly was controlled by, in part, body-wall receptors. If these receptors are present in *C. inornata*, the pressure created in the abdominal cavity by the ingestion of fluids would activate them and bring about the elevation of the threshold by sending inhibitory impulses from the receptors to the central nervous system. Owen (personal communication), and Dethier and Gelperin (1967) showed that by severing the ventral nerve cord it was possible to produce hyperphagia in *C. inornata* and *P. regina* respectively.

From the foregoing discussion it is concluded that probably in *C. inornata* the labellar threshold of response to sucrose is regulated by the same mechanism as in the blowfly; namely, via recurrent nerve, body-wall stretch receptors and ventral nerve cord.

ACKNOWLEDGMENT. The author wishes to express his sincere thanks to Dr. William B. Owen, Department of Zoology and Physiology, The University of Wyoming, for his guidance throughout this study.

References

Arab, Y. M. 1959. Some chemosensory mechanisms in the blowfly, *Phormia regina* Meigen. Bull. Coll. Sci. Baghdad Univ. 4:77–85.

Crow, Selma. 1932. The sensitivity of the legs of certain Calliphoridae to saccharose. Physiol. Zool. 5:16–35.

Dethier, V. G. and D. Bodenstein. 1958. Hunger in the blowfly. Z. F. Tierpsychol. 15:129–140.

Dethier, V. G. and A. Gelperin. 1967. Hyperphagia in the blowfly. J. Exp. Biol. 42:191–200.
Evans, D. R. and V. G. Dethier. 1957. The regulation of taste thresholds for sugars in the blowfly. J. Ins. Physiol. 1:3–17.
Fier, D. J., I. Lengy and W. B. Owen. 1961. Contact chemoreception in the mosquito, *Culiseta inornata* (Williston); sensitivity of the tarsi and labella to sucrose and glucose. J. Ins. Physiol. 12:829–841.
Gelperin, A. 1966. Investigation of a foregut receptor essential to taste threshold regulation in the blowfly. J. Ins. Physiol. 6:13–20.
Minnich, D. E. 1922. A quantitative study of tarsal sensitivity to solutions of saccharose in the red admiral butterfly, *Pyrameis atalanta* Linn. J. Exp. Zool. 36:445–457.

TOXICITIES OF THIOTEPA, TEPA, METEPA AND HEMPA TO LATE AQUATIC STAGES OF *CULEX NIGRIPALPUS* THEOBALD [1]

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ABSTRACT. Toxicities of aqueous concentrations of four chemosterilants to 3rd and 4th instar larvae and pupae of *Culex nigripalpus* were determined, preparatory to attempts at chemosterilization. The LC_{50}'s (ppm) for the respective stages listed were 3.5, 3.75, and 8,300 for thiotepa; 9.5, 11.9, and 31,250 for tepa; 80, 103, and 9,700 for metepa; and 267, 360, and 8,500 for hempa. More than 90% of larvae were killed when treatment concentrations exceeded 15 ppm of thiotepa, 25 ppm of tepa, 150 ppm of metepa, and 800 ppm of hempa.

INTRODUCTION. The mosquito, *Culex nigripalpus* Theobald, was shown to be the most important vector of St. Louis encephalitis in the Florida counties surrounding Tampa Bay during the epidemic of 1962 (Chamberlain *et al.*, 1964; Dow *et al.*, 1964). Previously it had not been known to be associated with epidemics of this disease. Probably this mosquito becomes important only under certain favorable epidemiological and ecological situations which favor its abundance, its infiltration of suburban sections, and the presence of susceptible hosts. The species is found in the southeastern United States, Mexico, Central, and northern South America.

Due to its low numbers during the late winter and spring (Provost, 1969), the possibility of effecting a high degree of control or near eradication in localized areas at that time by application of sterility techniques appeared feasible. This view was supported by the eradication of a related species, *Culex pipiens quinquefasciatus* Say, by dissemination of sterilized males in a recent demonstration (Patterson *et al.*, 1970).

Thiotepa, tepa, metepa, and hempa are known to be effective chemosterilants of related species (Saito and Hayashi, 1967; Pillai and Grover, 1969; Patterson *et al.*, 1970). As no chemosterilants had been tested on *C. nigripalpus*, toxicological data on stages believed most susceptible to treatment with the four chemosterilants chosen was desirable.

MATERIALS AND METHODS. All specimens tested were from a laboratory colony of *C. nigripalpus* maintained at the University of Florida mosquito laboratory since March, 1970. This colony was established from egg rafts obtained from the State Board of Health's West Florida Arthropod Research Laboratory at Panama City, Florida.

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[1] This research was supported by Public Health Service research grant CC 00366, from the Center for Disease Control, Atlanta, Georgia.

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