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REGULATION OF BLOOD MEAL SIZE IN THE MOSQUITO*

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Abstract—In order to determine the rôle played by the central nervous system in regulating blood meal size, the ventral nerve cord was cut at various sites along its length in the female mosquito, *Aedes aegypti.* When the cord was cut anterior to the second abdominal ganglion, massive hyperphagia resulted, many females ruptured, and blood intake was more than four times that of the untreated controls. As the site of the operation was moved stepwise posteriorly leaving more ganglia connected to the brain via the ventral nerve cord, the degree of hyperphagia and quantity of ingested blood was reduced. On the basis of these observations it was concluded that the termination of feeding was initiated by segmental abdominal stretch receptors which act in concert and signal the presence of optimal blood meal volume to the brain.

Five additional mosquito species were tested for hyperphagia and all ingested three to four times the normal blood meal volume when the ventral nerve cord was cut. Females with the nerve cord severed showed normal ovarian development and egg formation, but oviposition was inhibited.

INTRODUCTION

Because of the relationship of blood feeding to the vectorial capacity of mosquitoes, this aspect of mosquito biology has received a great deal of attention. Host sensing, feeding, blood meal size, and digestion have been considered by a number of authors and these topics have been thoroughly reviewed by Clements (1963). Although maximum blood meal size has been determined for a number of mosquito species, the intrinsic factors which regulate the quantity of blood ingested have not been ascertained. This investigation is concerned with the rôle of the central nervous system in regulating total blood meal volume.

MATERIALS AND METHODS

Table 1 lists the species of mosquitoes used in this study. Unless specified, all experiments were conducted with the Rockefeller Institute (ROCK) strain of *Aedes aegypti.* ROCK is a relatively large, uniform, vigorous strain used in a number of laboratories and may be considered as typical of the type form of *A. aegypti aegypti.*

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All rearing was conducted in accordance with the methods of CRAIG and VANDEHEY (1962). Optimum rearing conditions were maintained at all times. Crowding of larvae was avoided and only females which pupated within 6 days of hatching were utilized. Animals were maintained at 27 ± 1°C and 80 ± 5 per cent r.h.

TABLE 1—LABORATORY STRAINS OF MOSQUITOES USED IN DETERMINING MECHANISMS WHICH REGULATE BLOOD MEAL SIZE

Species	Source*
Aedes (*Stegomyia*) *aegypti* (L.)	Rockefeller Institute strain, maintained in this laboratory since 1959
A. (*Ochlerotatus*) *taeniorhynchus* (Wiedemann)	Donald Weidhaas, U.S.D.A., Gainesville, Fla.
A. (*Finlaya*) *triseriatus* (Say)	Herbert Schoof, U.S.P.H.S., Savannah, Ga.
Armigeres subalbatus (Coquillett)	A. R. Barr, University of California at Los Angeles
Culex pipiens fatigans Wiedemann	D. L. Shankland, Purdue University, Ind.
Anopheles quadrimaculatus Say	Donald Weidhaas, U.S.D.A., Gainesville, Fla.

* All species now maintained at Vector Biology Laboratory, University of Notre Dame, Indiana.

Operating procedures

In order to determine the rôle played by the central nervous system in regulating blood meal size, the ventral nerve cord was cut at various points along its length in the abdomen. Virgin females, 3 to 5 days old, were anaesthetized with ether and placed ventral side up on a piece of modelling clay. The body was held in place with a strip of clay across the thorax and the legs were spread with hooked minutien pins. The animals were then flooded with saline (HAYES, 1953). Dissection was performed under a 140 × stereoscope.

To expose the ventral nerve cord anterior to the second abdominal ganglion, an incision was made across the membranous area between the thorax and the second abdominal sternite. Severing the cord at this point isolated all the abdominal ganglia with the exception of the first, which is fused with thoracic ganglionic mass. An incision between sternites 2 to 3, 3 to 4, 4 to 5, or 5 to 6 permitted the cutting of the ventral nerve cord anterior to abdominal ganglia numbers 3, 4, 5, or 6 respectively. Sham-operated females were handled in like manner, with an incision across the membranous area posterior to the thorax, but with the cord left intact.

After the operation, females were transferred to individual 80 × 25 mm shell vials with moist cotton plug bottoms. The procedure took less than 5 min/female and mortality after 48 hr was less than 5 per cent. After the 48 hr recovery period, mosquitoes were fed individually on the forearm of the author. Females with the nerve cord severed showed normal ovarian development and egg formation; however, these females never oviposited. Feeding time was recorded as the time from insertion to withdrawal of the fascicle. Blood meal quantity was determined by weighing females immediately after feeding. Egg development was based on dissections and counts of individual females 72 hr after feeding. Feeding time, weight, and egg development were used as indicators of the effects of the serial cutting of the ventral nerve cord.

For the species other than *A. aegypti*, the ventral nerve cord was cut anterior to the second abdominal ganglion. The animals were held for 48 hr, fed, and the quantity of ingested blood determined. Feeding time and egg production were not recorded although eggs were developed by all females that fed.

RESULTS

When the ventral nerve cord was cut anterior to the second abdominal ganglion, females took unusually large blood meals. Indeed, hyperphagic females would often continue to suck blood until the abdomen ruptured. Fig. 1 shows untreated and hyperphagic females of *A. aegypti* and *Anopheles quadrimaculatus*.

Table 2 shows the effect of cutting the ventral nerve cord at various sites in females of *A. aegypti*. In all cases, feeding appeared to be normal, albeit prolonged, and no obvious post-operative damage was noted. When the ventral nerve cord was cut posterior to the second ganglion, no ruptures occurred.

TABLE 2—EFFECT OF CUTTING THE VENTRAL NERVE CORD AT VARIOUS SITES ON FEEDING TIME, BLOOD MEAL SIZE, AND EGG DEVELOPMENT IN FEMALE *A. aegypti*

Treatment: Ventral nerve cord cut anterior to abdominal ganglion	No. females/ treatment	Mean for treated females (± 95% confidence limits)		
		Feeding time (min)	Blood meal weight (mg)	No. of eggs developed
No. 2	76	5·31 ± 0·24	11·99 ± 0·51	103·28 ± 4·31*
No. 3	42	3·21 ± 0·32	7·63 ± 0·70	97·17 ± 4·01
No. 4	36	2·69 ± 0·22	5·14 ± 0·40	92·32 ± 2·81
No. 5	40	2·19 ± 0·16	4·23 ± 0·46	88·88 ± 4·25
No. 6	36	1·98 ± 0·18	3·61 ± 0·21	87·14 ± 4·39
Sham-operated†	62	1·76 ± 0·11	2·93 ± 0·18	85·78 ± 3·03
No treatment‡	64	1·79 ± 0·10	2·85 ± 0·26	86·07 ± 3·30

* Mean for 25 females which survived 3 days and developed eggs. For all other treatments, survival to day 3 was greater than 95 per cent.

† Pre-feeding weight 2·11 ± 0·07 mg.

‡ Pre-feeding weight 2·61 ± 0·14 mg.

As indicated in Table 2, animals with a severed ventral nerve cord ingested a significantly larger quantity of blood. For females which had been cut anterior to the second ganglion, the increase was fourfold. As the site of the operation was shifted posteriorly from ganglion to ganglion, the quantity of ingested blood showed a significant decrease. From the second to the third ganglion the decrease was approximately 4·5 mg, whereas from the third to the fourth the decrease was 2·5 mg. For the remainder of the sites, the decrease in the amount of ingested blood was less pronounced, but still significant. Feeding time and number of eggs developed showed a definite decrease as the site of the operation was moved from the second to the sixth ganglion, although the change was less dramatic.

Table 3 lists data for five species of mosquitoes, in addition to *A. aegypti*, that were tested for hyperphagia by cutting the nerve cord. In all cases, the treated females were hyperphagic and the quantity of ingested blood was at least three to four times that of the untreated females. The feeding of *A. quadrimaculatus* was interrupted after 3 min; females of this species invariably ruptured when allowed to feed longer. Females of *Aedes taeniorhynchus* did not rupture when allowed to feed to repletion, although over 25 per cent of the females of the other four species ruptured as a result of hyperphagia.

TABLE 3—EFFECT OF CUTTING THE VENTRAL NERVE CORD ANTERIOR TO ABDOMINAL GANGLION No. 2 ON BLOOD MEAL SIZE IN SIX DIVERSE SPECIES OF MOSQUITOES

Species	No. females/ treatment	Pre-feeding weight (mg)	Blood meal weight (mg) No treatment	Blood meal weight (mg) Ventral nerve cord cut
Aedes aegypti	76	2·61 ± 0·14	2·85 ± 0·26	11·99 ± 0·51
A. taeniorhynchus	10	3·11 ± 0·33	3·84 ± 0·65	15·91 ± 0·99
A. triseriatus	10	2·79 ± 0·18	3·93 ± 0·17	17·19 ± 1·24
Armigeres subalbatus	10	3·62 ± 0·33	3·74 ± 1·46	16·52 ± 1·50
Culex pipiens fatigans	10	2·87 ± 0·24	4·02 ± 0·30	13·44 ± 2·49
Anopheles quadrimaculatus	12	1·91 ± 0·34	2·33 ± 0·38	9·82 ± 1·03*

* Females with cut ventral nerve cord allowed to feed for 3 min. Other species allowed to feed to repletion.

DISCUSSION

Blood components which induce engorgement by feeding mosquitoes have been considered by a number of authors (HOSOI, 1959; GALUN *et al.*, 1963), while the sensory mechanisms which determine food distribution to the midgut or crop have also received considerable attention (TREMBLEY, 1952; DAY, 1954; HOSOI, 1954). However, the mechanisms which sense blood meal volume and initiate the termination of feeding have not been elucidated.

WIGGLESWORTH (1934) noted the presence of stretch receptors in the abdominal wall of *Rhodnius prolixus*, and their connexion via the ventral nerve cord to the brain. Although he did not investigate their possible control of blood meal size, he did establish the relationship of these receptors, the ventral nerve cord, and the presence of a blood meal to the initiation of pre-moult hormone secretions. On the

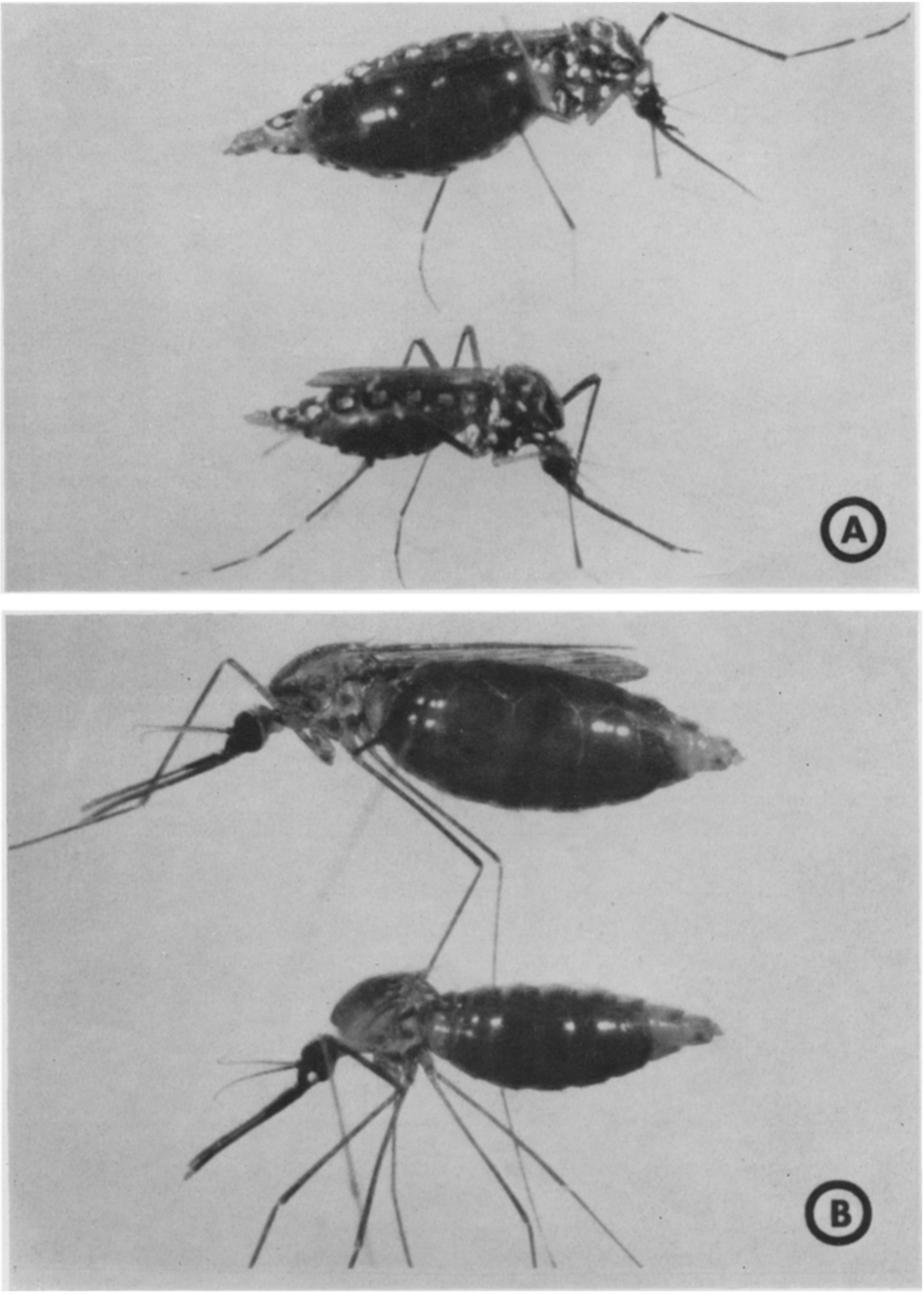

FIG. 1. Females of *Aedes aegypti* (A) and *Anopheles quadrimaculatus* (B). The ventral nerve cord of the upper female in each pair was cut anterior to the second abdominal ganglion. These females were allowed to feed for 3 min and were then interrupted. The lower female of each pair, which was untreated, was allowed to feed to repletion; it represents the normal post-feeding appearance.

other hand, Bennet-Clark (1963), also with *Rhodnius prolixus*, attempted to show that blood meal size was controlled simply by the mechanical expansion properties of the abdominal cuticle.

Hyperphagia, induced by nerve transection, has been demonstrated in the flies *Phormia regina* (Dethier and Bodenstein, 1958; Dethier and Gelperin, 1967) and *Lucilia* sp. (Nuñez, 1964). Nuñez produced hyperphagia by cutting the ventral nerve cord between the brain and the thoracic ganglionic mass. Dethier and Gelperin performed operations on the ventral nerve cord anterior to the thoracic ganglionic mass, on abdominal body wall nerves extending from the thoracic mass, and on the recurrent nerve. In all cases, hyperphagia resulted when the treated flies were allowed to feed on sugar solutions. It was their conclusion that abdominal stretch and proprioreceptors had some rôle in the normal termination of feeding (Dethier and Gelperin, 1967). They felt, however, that the stomatogastric nervous system, involving connexion to foregut stretch receptors, was equally important.

Hyperphagia in *Phormia* and *Lucilia* differs considerably from that in mosquitoes. Not only are the former sugar feeders, but the storage site for food in these species is the crop. Indeed, even among the blood-sucking higher Diptera such as *Glossina* and *Stomoxys*, the storage site of blood is the crop.

In mosquitoes, although sugar solutions are stored in the crop, blood goes directly to the midgut (Trembley, 1952).

The morphology of the abdominal nervous system of mosquitoes is considerably different from that of the higher Diptera or *Rhodnius*. In the latter animals, the abdominal ganglia are consolidated and fused in the thoracic ganglionic mass. In adult mosquitoes, the segmental ganglionic arrangement is preserved, with individual ganglia in the second to sixth abdominal segments and the seventh and eighth ganglia fused in the seventh segment.

Because of this segmental ganglionic arrangement, the presence of segmental abdominal stretch receptors can be readily demonstrated. As shown in Table 2, the isolation of the second to seventh ganglia produces massive hyperphagia. However, if the second abdominal ganglion remains connected via the ventral nerve cord to the brain, hyperphagia is markedly reduced although still pronounced. As the site of the operation is moved posteriorly and more ganglia are left in line with the brain, hyperphagia is reduced stepwise. If only the sixth and seventh ganglia are isolated, the quantity of blood ingested approaches normal although it is still significantly larger.

The cord was not cut between the sixth and seventh ganglia because this operation usually results in damage to the last ganglion. With the terminal ganglion removed or damaged, defaecation is inhibited, and at 48 hr after surgery females are very reluctant to take a blood meal.

It would appear, from the above data, that termination of feeding is not initiated by a single sense organ. The segmental arrangement of ganglia and stretch receptors seems to exert a cumulative effect as the midgut swells with blood.

Larsen and Bodenstein (1959) hypothesized that expansion of the midgut of the mosquito following a blood meal provided the initial stimulus for egg maturation. They felt that this stimulus was nervous in nature and activated neurosecretory centres in the brain, leading eventually to egg development. They theorized that this impulse was transmitted via the recurrent nerve of the stomatogastric system.

In *Rhodnius*, gut distension intitiates brain hormone secretion which produces a moult. The nervous impulse is transmitted from the abdomen to the brain via the ventral nerve cord (WIGGLESWORTH, 1934). If the mosquito system were analogous to that of *Rhodnius*, one could expect ovarian development to be initiated in the same way. Indeed, this analogy has already been drawn and incorporated into the popular literature. JONES (1968) states that following a blood meal 'the gut presses on the ventral nerve cord and signals are sent to the brain', hormones are secreted, and ovarian development begins. As previously noted, severing the ventral nerve cord may have produced hyperphagia, but it did not interfere with ovarian development.

If gut distension does indeed produce a nervous impulse which triggers hormone secretions, the stomatogastric system is the more likely pathway.

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