THE MECHANISM OF FOOD DISTRIBUTION TO MIDGUT OR DIVERTICULA IN THE MOSQUITO

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Summary

An examination has been made of factors involved in the despatch of sugars to the diverticulum and blood to the midgut in the mosquito. It is suggested that sense organs observed in the buccal cavity of the mosquito detect the presence of some sugars and components of blood. The resulting nervous impulse is probably transmitted via the stomatogastric nervous system to cause the contraction of sphincter muscles of the diverticula or the proventriculus. The significance of this switching mechanism in mosquito feeding is discussed.

I. INTRODUCTION

A knowledge of how mosquitoes transmit virus diseases requires an understanding of their processes of ingestion and digestion. One of the peculiarities of mosquito digestion that has been known for a long time is that, whereas whole blood passes directly to the midgut, solutions of sugars generally pass to the diverticula, rather than to the midgut. The problem of the distribution of liquids to these regions of the mosquito gut has recently been studied by Trembley (1952). Her main conclusions, incorporating some of those of earlier workers whom she quotes, may be summarized as follows:

(1) Blood, irrespective of the manner in which it is fed, goes primarily to the midgut. In some species practically none reaches the diverticula, in others a considerable amount of blood may go to them.

(2) Solutions of sugars (glucose or honey) go primarily to the diverticula, mainly the ventral diverticulum. Traces may go to the midgut at once, but normally the contents of the ventral diverticulum are later passed to the midgut, a small amount at a time.

The establishment of these facts does not answer the question of how various liquids reach the respective regions of the gut and, in fact, no comprehensive suggestion has been advanced that does provide an explanation of what Fisk (1950) has called the "switching mechanism." Boissezon's (1930) suggestion that the diameter of the ducts to the diverticula was not great enough to permit the passage of erythrocytes does not account for all of the observations, and Marshall and Staley's (1932) conclusions concerning the action of sphincters at the openings of the diverticula offer no indication of how these sphincters are activated. Fisk (1950) performed some experiments to determine what properties of blood enabled it to reach the midgut, but stated that his results did not solve the problem.

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Consideration of the conclusions of Trembley referred to above and of the anatomical relationships of the gut of the Diptera suggested that sense organs located in or near the buccal cavity may be stimulated by components of the food, resulting in the transmission of motor impulses to the sphincters of the diverticula or the proventriculus, or both.

Attempts to obtain data to evaluate this suggestion are reported in this paper. The results permit the formulation of a hypothesis that appears to provide an explanation of the "switching mechanism."

II. Materials and Methods

Of the species of mosquitoes examined by Trembley (1952), *Aedes aegypti* (L.) appeared to exhibit the switching mechanism to a marked degree, and it was therefore suitable for this work.

As the method of feeding does not influence the destination of liquids ingested (Bishop and Gilchrist 1946; Trembley 1952), heparinized rabbit blood and blood and sugar mixtures were fed to starved *A. aegypti* from cotton wool or filter paper soaked in the fluid. Liquids other than blood were generally coloured with carmine. Mosquitoes, kept overnight in small glass vials covered with mosquito netting, could frequently be stimulated to ingest blood and sugar mixtures by having them briefly probe the skin of the forearm and immediately removing them to small dishes (containing the cotton wool and substances to be tested) standing on a thermostatically controlled warm plate. Difficulty was experienced in getting numbers of mosquitoes to accept some of the mixtures, particularly those lacking erythrocytes. Larger numbers of fed mosquitoes of both sexes were obtained in several instances by collecting insects as they fed from saturated filter papers in dishes containing the blood-sugar mixtures. Dissections were performed in a drop of *Drosophila* Ringer.

Ten-micron serial sections of mosquitoes stained with Mallory's triple connective stain or by Bodian's protargol method were used to study the sense organs. The anatomy, innervation, and musculature of the gut were studied also in whole mounts of the gut after staining with methylene blue by the method of Kuwana (1935). Phase-contrast microscopy, using a Leitz Heine condenser, was used to study the crop musculature and innervation.

III. Feeding Experiments

Although several earlier authors have reported the destination of mixtures of blood and sugars in the mosquito gut, the data are inadequate for the present purposes. Experiments were therefore performed to determine what components of the blood or blood : sugar mixtures were stimulatory and what were the approximate thresholds for stimulation. The results (Table 1) demonstrate that, in agreement with the work of earlier authors, blood reaches the midgut whereas glucose solutions go mainly to the diverticula.* The addition of a

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* Frequently liquids were found only in the ventral diverticulum, whereas the dorsal diverticula contained gas bubbles. Only the ventral diverticulum was considered in the results presented.

small amount of blood to 10 per cent. glucose solution results in the mixture going partly to the midgut and partly to the diverticulum. Thus 1 part of blood to 20 of glucose was sufficient to be detected and to activate the switching mechanism. Over a wide range of concentrations of blood in mixtures of glucose solutions the mixture went both to the midgut and to the diverticulum.

TABLE 1

DESTINATION OF MIXTURES OF 10 PER CENT. GLUCOSE AND WHOLE BLOOD WHEN INGESTED BY
AEDES AEGYPTI

Composition of Liquid	Molar Concn. of Glucose	Number Engorged	Destination (%)	
			Diverticulum	Midgut
Blood	—	50	6*	100
Blood 3 ml; glucose 1 ml	0·08	25	16	92
Blood 2 ml; glucose 1 ml	0·13	20	40	70
Blood 1 ml; glucose 1 ml	0·25	30	73	80
Blood 0·5 ml; glucose 1 ml	0·30	30	83	86
Blood 0·25 ml; glucose 1 ml	—	20	80	90
Blood 0·1 ml; glucose 1 ml	0·46	30	100	7
Blood 0·05 ml; glucose 1 ml	0·50	25	100	8
Glucose 1 ml	—	50	100	10

* Trace only. (Note that particularly at intermediate concentrations the ingested liquid frequently goes to both midgut and diverticulum in the same insect.)

If glucose is added to heparinized blood in sufficient concentration, the mixture again goes both to the midgut and diverticulum. The conclusion is inescapable that both whole blood and glucose are detected, and the relative concentration of the components of a mixture determines its destination. These results suggested the search for separate receptors to detect glucose and blood (see Section IV below).

The experiment was repeated replacing glucose by other sugars. Equal parts of 5 per cent. sucrose and heparinized blood went mainly to the diverticulum, indicating that sucrose was detected (Table 2). On the contrary, a mixture of 2 ml 10 per cent. lactose in 1 ml heparinized blood went entirely to the midgut, suggesting that lactose was unable to stimulate the receptors. Insufficient numbers of mosquitoes for a study of thresholds could be induced to accept blood mixed with arabinose, mannose, and raffinose, but all three could apparently be detected, for when these sugars were mixed with blood at a concentration of 0·2 g/ml the mixture went both to the diverticulum and to the midgut.

Experiments were next designed to determine what components of whole blood were responsible for the switching mechanism. Similar experiments performed by Fisk (1950) did not permit conclusions to be drawn from them,

but Bishop and Gilchrist (1946) found that plasma, washed erythrocytes, and haemolysed blood when ingested went to the midgut and not to the diverticulum. These data and the results presented in Table 2 suggest that both plasma and erythrocytes are detected but the reaction to plasma is less marked than that to washed erythrocytes. Solutions of haemoglobin and albumin with glucose went mainly to the diverticulum, and were thus presumably not detected; however, these substances were ingested by too few mosquitoes for the results to be unequivocal.

TABLE 2

DESTINATION OF MIXTURES OF SUGARS AND BLOOD AND BLOOD CONSTITUENTS WHEN INGESTED BY
AEDES AEGYPTI

Composition of Liquid	Number Fed	Destination (%)	
		Diverticulum	Midgut
Blood 5·0 ml; 3·5% sucrose 1·0 ml	20	0	100
Blood 0·3-0·2 ml; 3·5% sucrose 1·0 ml	20	100	100
Blood 1·0 ml; 5% sucrose 1·0 ml	20	100	10
Erythrocytes* 0·3 ml; 5% glucose 1·0 ml	20	50†	100
Erythrocytes* 0·1 ml; 5% glucose 1·0 ml	20	100	20‡
Plasma§ 1·0 ml; 5% glucose 1·0 ml	20	100	45
Plasma§ 1·0 ml; 5% sucrose 0·5 ml	20	80	30
Plasma	20	100	20

　* Prepared by twice centrifuging heparinized rabbit blood and resuspending the sedimented cells in physiological saline to the original volume.

　† In three of the 10 insects of the group, solution, but no erythrocytes were present in the diverticulum.

　‡ Erythrocytes present in midgut only, none in diverticulum.

　§ Supernatant from first centrifugation of * above.

When mixtures of washed erythrocytes and glucose were fed to mosquitoes, the mixtures went to the midgut, the diverticulum, or to both, depending upon the concentrations of the components. When the concentration of washed erythrocytes was low, however, a number of mosquitoes were found to have diverticula filled with a clear liquid, whereas the midguts contained liquid together with a few erythrocytes. The straining mechanism mentioned by Boissezon (1930) appears to be effective in these instances. The occurrence of erythrocytes in the diverticulum when blood : sugar mixtures in other concentrations are fed demonstrates, however, that this straining apparatus is not normally able to control the switching mechanism.

A group of spines was observed in the neck of the ventral diverticulum (Plate 1, Fig. 5). These do not seem to have been noticed by previous workers; they differ in shape and arrangement from those described in the pyloric region of the same species by Trembley (1951). Their presence would assist in keeping erythrocytes out of the diverticulum when the circular muscles at the neck

were contracted, and they probably function in straining out the erythrocytes as described in the previous paragraph.

The response to washed erythrocytes suggested that the stimulus might be tactile rather than gustatory. An attempt to examine this possibility was made by feeding mosquitoes on washed red cell ghosts. Rabbit erythrocytes were haemolysed in distilled water, centrifuged, resuspended in distilled water, and centrifuged again. The resulting ghosts, pinkish in colour, were resuspended in a minimum of water and fed to 7-day-old, previously unfed mosquitoes. Five ingested these ghosts and in all the suspension went mainly to the midgut. Thus washed red cell membranes, though less readily accepted than erythrocytes, were able to activate the switching mechanism.

It was possible that residual haemoglobin may have been responsible for causing the membranes to be despatched to the midgut; it was therefore considered desirable to determine the destination of other particulate material after ingestion by the mosquito. Several such materials (water-insoluble dyes, starch grains) were not ingested. Finally, starved mosquitoes did ingest washed sarcosomes separated from the thoracic muscles of the blowfly *Lucilia cuprina* (Wied.). When suspended in distilled water, these approximated the diameter of mammalian erythrocytes. In 20 *A. aegypti* females that ingested this suspension, the material was found both in the midgut and the diverticulum. The supernatant from a centrifuged preparation went only to the diverticulum. It seems likely, therefore, that it was the particulate nature of the sarcosomes that caused some of the suspension to be despatched to the midgut.

In some experiments males were used in addition to females. The two sexes reacted similarly; blood went to the midgut and sugar to the diverticulum in the male in spite of the fact that males do not ingest blood under natural conditions.

In some early experiments it seemed possible that the age of the mosquitoes or their previous feeding were responsible for variations in their behaviour with respect to the destination of blood : sugar mixtures. A series of tests was therefore set up to determine whether these factors were contributing to the variability of behaviour. In one test comparisons were made between 2-day-old females previously unfed and 7-day-old females that had had access to sugar water for the first 6 days after emergence. There was no difference in the destination of blood : glucose mixtures in the two groups. In a second test no difference in the destination of blood : glucose mixtures was detected in a comparison between females that had had no previous feed and females that had had a single blood meal 7 days previously. Variability in destination occurred equally in all groups, and no method was developed which resulted in completely uniform behaviour of any groups of insects towards blood : glucose mixtures. There was much more marked uniformity, however, in response to sugar or to whole blood offered separately.

IV. Anatomical Considerations

The feeding experiments reported in Section III suggested that two types of sense organs might be necessary to detect both sugars and the components

of blood.   Although pharyngeal sense organs have been recorded in several
insects (e.g. in larval *Drosophila* (Hertweck 1931), *Glossina* (Jobling 1933), and
in *Panorpa* (Grell 1938)), no record of their occurrence in mosquitoes could
be found.   Examination of the stylets showed the absence of appropriate re-
ceptors in these chitinous structures (see also Robinson 1939).   Serial sections
of the head of *Aedes aegypti* revealed the presence of the dorsal and palatal
papillar sense organs in the buccal cavity.   These were first described by Sinton
and Covell (1927), and by Barrand and Covell (1927), who showed that their
form and arrangement had considerable taxonomic value.   The location and
arrangement of these sense organs in *Aedes aegypti* is shown in Figures 1 and
2.   Sections showed that the sense organs were of four types, as illustrated in
Plate 1, Figures 1-4; the three pairs of palatal organs were spines, whereas the
three pairs of dorsal organs were hair-like receptors and there was a single pair
of campaniform papillae.   Similar structures were found in male *A. aegypti*.
The cibarial sense organs described in adult *Drosophila* by Hertweck (1931)
and Miller (1950) are probably homologous with these papillar sense organs
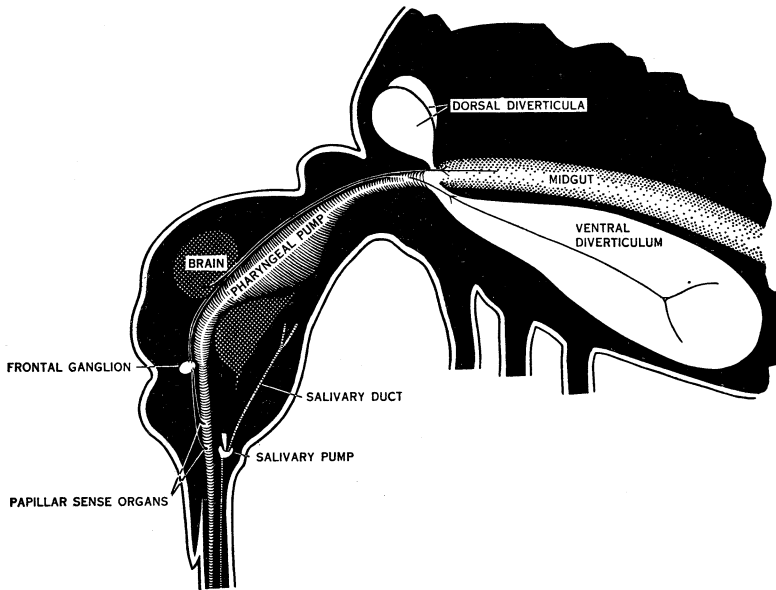of mosquitoes.

Fig. 1.—Diagrammatic median longitudinal section of anterior part of a
mosquito, showing foregut and midgut and the stomodeal nervous system.

    The precise function of the papillar sense organs cannot, of course, be cer-
tainly adduced from their structure; but the form of the palatal papillar organs
is not inconsistent with the suggestion that they are chemoreceptors; the struc-
ture and position of the dorsal papillar receptors suggest that they may be
capable of detecting the flow of erythrocytes and of the sarcosomes mentioned
in the previous section.   One of the four types of sense organ is apparently
able to detect the presence of blood plasma.

The innervation of the seven pairs of sense organs could not be traced with certainty, but all received nerves from the frontal ganglion. (Miller (1950) believed also that the pharyngeal nerves that innervate the cibarial sense organ of *Drosophila* are homologous with the frontal ganglion connectives, and Wenk (1953) has shown that the labral nerves of the flea *Ctenocephalus* innervate cibarial sense organs.) It therefore seems likely that nerve impulses from the mosquito sense organs reach the stomatogastric nervous system. In all insects the stomatogastric nervous system innervates the foregut as well as the incretory organs of the head. This has been well shown in *Calliphora* (Graham-Smith 1934), and a similar arrangement has been revealed in favourable methylene blue preparations of *Aedes aegypti*. Orlov (1924) has shown that the frontal ganglion, of *Oryctes* larvae at least, contains sensory as well as motor nerve cells. The origin of these cells has never been elucidated. The experiments of Bolwig (1952) suggest that the frontal ganglion is not concerned with peristaltic movements in the fly gut and its function is not completely understood.
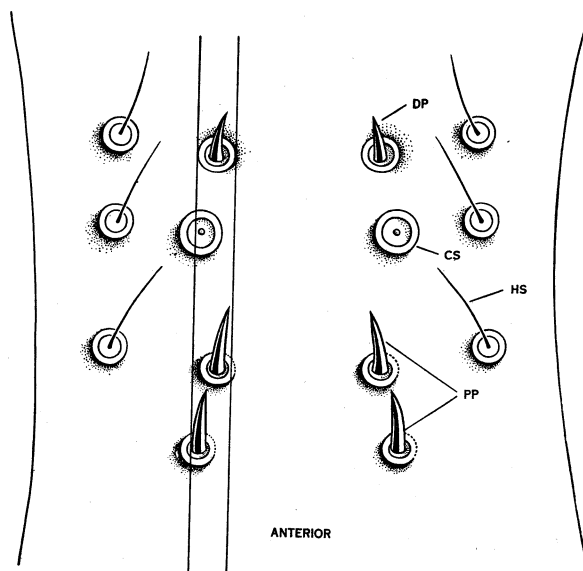

Fig. 2.—Diagram of number and arrangement of sense organs in the dorsal wall of the buccal cavity of *Aedes aegypti*. Palatal papillar sense organs (*PP*); campaniform sensillum (*CS*); dorsal papillar sense organ (*DP*), and hair-like sensilla (*HS*). The parallel lines indicate the plane of the section of Plate 1, Figure 4.

The innervation of the diverticula of *Aedes* is shown semi-diagrammatically in Figure 1. The arrangement of the musculature in an expanded ventral diverticulum and the relative size of the nerve and its method of branching are shown in Plate 1, Figure 6.

The origin of the nerves innervating the muscles of the diverticula is still not definitely known, but it seems most likely that they form part of the stomatogastric nervous system. It is not essential for the present purposes to know the precise neural pathway between the sensory receptors and the effector system. It is entirely possible that some central connections exist. However, the pathway through the frontal ganglion and the stomatogastric nervous system appears possible and is the simplest way of accomplishing the connections.

V. Discussion

The following hypothesis to explain the switching mechanism in the mosquito is suggested by the data presented above. Certain sugars stimulate the pit organs in the buccal cavity of the mosquito, causing impulses that result in the relaxation of the sphincters of the diverticula. Certain components of the blood of vertebrates stimulate the papillar sense organs, resulting in relaxation of the cardiac sphincters. Simultaneous stimulation of both groups of sense organs may result in relaxation of both series of sphincters. Both male and female mosquitoes react in the same manner to these ingested materials, even though the males never obtain a blood meal under natural conditions.

The thresholds of the pit organs to sucrose and glucose are somewhat high in comparison with tarsal and palpal receptors (see Dethier 1953) but these receptors would respond to the concentration of sugars normally found in nectars.

The function of the diverticula as containers of ingested sugar solutions does not, of course, preclude them from having additional functions. In some of the present experiments they were found to function also as "air separators" as previously shown by MacGregor (1930).

The question may be raised of the survival value of the switching mechanism in the mosquito and of the absence of such a mechanism in, for example, the blowfly, in which the necessary anatomical requirements would appear to be well developed. It is suggested that the answer is to be found in the differences in the feeding behaviour of the two Diptera. In the majority of species female mosquitoes require a blood meal in order to mature their eggs. The opportunity to bite a suitable vertebrate to provide the blood meal will come at irregular intervals and the insect must be capable of utilizing the opportunity when it is presented. Nectar is more generally available and is sufficient to keep the insect alive. But the ability to take a blood feed in spite of a recent nectar meal would seem to be of survival value, and this is what is accomplished by the "switching mechanism"; a crop containing some nectar does not preclude the taking of a blood meal should this become available.

Trembley (1952) has shown that the switching mechanism is not as well developed in some species of mosquitoes as it is in *A. aegypti*. It may be that the species of *Anopheles* and *Culex* that she studied, in which erythrocytes were often found in the diverticula as well as the midgut, have either less sensitive receptors in the buccal cavity, or a less well-developed sphincter musculature in the necks of the diverticula.

FOOD DISTRIBUTION IN THE MOSQUITO
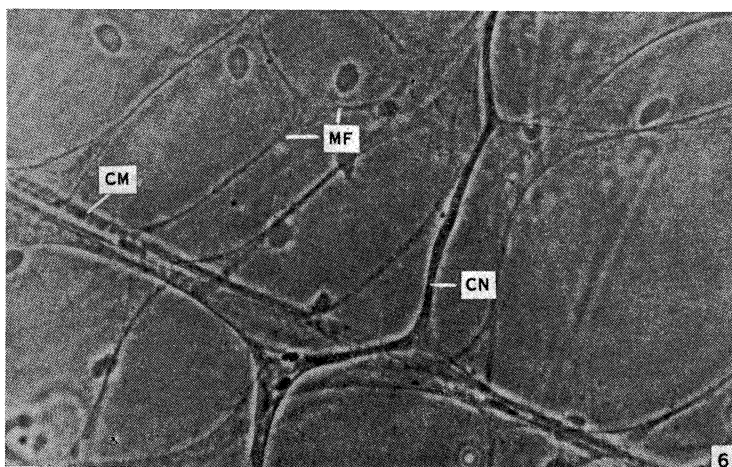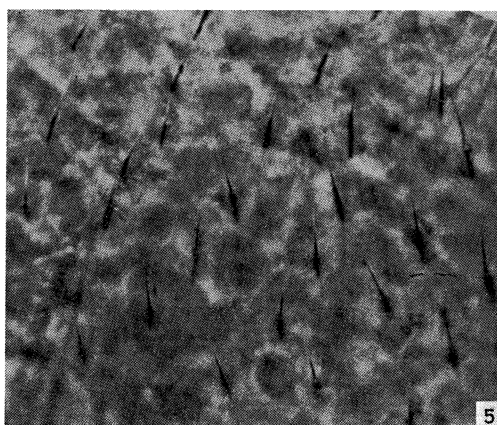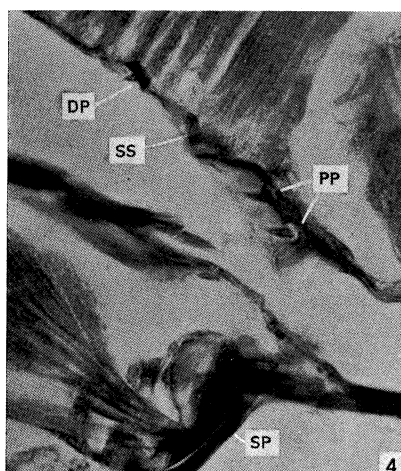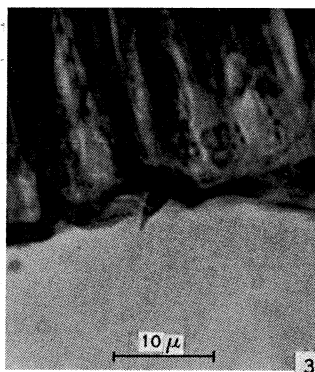
VI. Acknowledgments

VII. References

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Explanation of Plate 1

Photomicrographs of *Aedes aegypti*.

Fig. 1.—Longitudinal section of dorsal wall of buccal cavity showing structure of the palatal papillar sense organ projecting into the lumen. The spine is directed posteriorly.

Fig. 2.—The same, showing one of the six hair-like sensillae.

Fig. 3.—The same, showing structure of a dorsal papillar sense organ.

Fig. 4.—Longitudinal section of the buccal cavity, showing two palatal papillar sense organs (*PP*), a campaniform sensillum (*CS*), and a dorsal papillar sense organ (*DP*). The salivary pump (*SP*) is also shown. The anterior end of the insect is to the right of the illustration.

Fig. 5.—Whole mount of neck of ventral diverticulum, showing spines. These vary in length from about 15 to 30 μ. Phase contrast.

Fig. 6.—Whole mount of expanded crop showing arrangement of circular muscles (*CM*) and irregular network of fine muscle fibres (*MF*) connecting them and the crop nerve (*CN*), and its method of branching. The dark oval bodies are nuclei of the epithelium. Phase contrast.