Reports

Effects of Absence of Saliva on Blood Feeding by Mosquitoes

Abstract. The salivary glands of *Aedes stimulans* (Walker) are the source of an antigen which produces typical bite reactions in men and laboratory rabbits. If the main salivary duct is cut, the reaction is not produced when the mosquito bites. Lack of saliva does not affect the intake or movement of blood into the mid-gut, nor does it prevent the development of eggs. The presence of an anesthetic component in saliva is suggested.

The salivary secretions of a parasitic animal have two important aspects: the effects of introduction of the saliva into the host tissues, and the influence of salivary secretions upon the feeding mechanism and subsequent digestive processes of the parasite.

When a mosquito feeds upon a mammalian host a wheal commonly develops around the puncture site; in man wheal formation is usually accompanied by erythema and irritation. Wheals may also be produced in laboratory rabbits in response to a series of bites received over a period of time (*1*). Controlled experiments with rabbits have established the fact that the reaction to mosquito bites is of a hypersensitive type, and therefore occurs in response to the injection of antigen (*1*).

It has been generally assumed that the source of this antigenic material lies in the salivary glands, but no direct evidence for this assumption has been recorded. The following technique was devised to determine if a bite, unaccompanied by salivary secretion, would produce the characteristic symptoms in a sensitized human host.

Aedes stimulans (Walker) occurs abundantly in eastern Ontario during the summer months, and this comparatively large species was selected for initial attempts to sever the main salivary duct leading to the hypopharynx. Females were captured as they attempted to feed on collectors in the field and also were reared from field-collected larvae. All adults were maintained on 0.1*M* sucrose until they were required.

To cut the salivary duct, the female was first anesthetized with carbon dioxide and placed on its back on a slide. Strips of plasticine were crossed over the abdomen to hold the insect in place and the proboscis was gently strapped out in a position which extended the neck.

A needle of tungsten wire (diameter 10 μ), sharpened to a fine point, was used to make a small incision into the anterior region of the neck, exposing the dark salivary duct. Posteriorly this duct divides into two branches which lead to the tri-lobed glands on each side (Fig. 1). The duct was cut in the anterior position with a sharpened sliver of tungsten wire. The operated mosquito was transferred to a vial and supplied with sucrose solution. Mortality rates were high, undoubtedly because of handling techniques and also the variation in age of mosquitoes caught in the field.

After a recovery period of 24 hours the operated mosquito and an unoperated control were offered a blood meal from the arm of a human subject. Many of the surviving operated mosquitoes did not attempt to bite; however, 12 individuals became fully engorged and gave clear results. The bite of a female whose salivary duct had been cut failed, in every case, to produce a wheal or irritation; the bites of control mosquitoes, in an adjacent area on the arm, produced the normal reaction. These experiments give very good evidence that the source of antigenic material is the salivary glands.

The technique described offers an opportunity for study of the effect of salivary secretions upon digestion and utilization of the blood meal, and also of the possible role of saliva as a lubricant during insertion of the mouthparts.

A robust survivor with the salivary duct cut appears to experience no difficulty in inserting the mouthparts into the skin of the host. This is not surprising since the salivary canal in the hypopharynx is enclosed throughout its length, and saliva does not have access to the mandibles and maxillae except at the tip of the proboscis. Movement of the blood from the mouthparts to the mid-gut appeared to be normal. An interesting comparison may be made with the feeding of *Glossina morsitans* Westw. and *G. tachinoides* Westw. after removal of the salivary glands (*2*). These operated tsetse flies fed readily but the ingested blood subsequently clotted in varying degrees in the proboscis, proventriculus, and crop. This, it was claimed, resulted from the absence of an anticoagulin normally secreted by the salivary glands.

Clotting of human blood has been shown to be prevented by addition of extracts of salivary glands of several species of *Anopheles*. Clotting time was not prolonged by extracts of glands of *Aedes aegypti* L., and other species of *Aedes, Culex,* and *Psorophora* (*3*). The effect of glandular extracts of *A. stimulans* on blood in vitro is not known.

Larsen and Bodenstein (*4*) have shown that the blood meal initiates a series of humoral activities in the brain and corpora allata of *C. pipiens* L. and

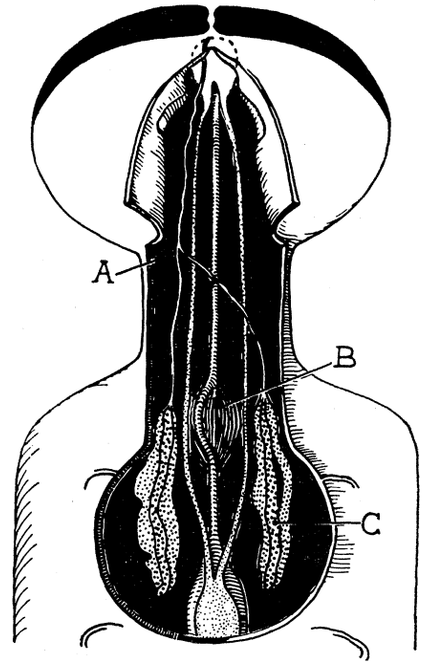

Fig. 1. Ventral view of the head, neck, and prothorax of a female *Aedes aegypti* mosquito dissected to show positions of the salivary ducts and glands. *A*, Branching of main salivary duct; *B*, proventriculus; *C*, left salivary glands.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

A. aegypti. This work has provided good evidence that it is the distension of the gut by the clotted blood which triggers a humoral chain of events culminating in maturation of the eggs.

We were interested in discovering if the absence of saliva affected the subsequent clotting of the blood in the mid-gut and if this would in turn affect egg development. The mid-guts of operated and blood-fed *A. stimulans* contain a blood clot which does not appear to be different, on examination under the microscope, from the clot formed in a normal mosquito. Moreover, the eggs develop to stages F and G (*4*), indicating that humoral activity has been initiated.

Recently we have been able to cut the salivary duct in *A. aegypti*, and experiments with mosquitoes of known age and diet should offer more accurate data. The results of the experiments are at present difficult to interpret in terms of the function of the salivary glands of mosquitoes. If saliva plays a part in digestion of the blood meal, this function may be reflected in the subsequent activities of the mosquito, rather than in the development of the current batch of eggs. It has been suggested (*5*) that the salivary glands of blood-sucking arthropods are now nonfunctional but remain as evidence of a plant-feeding ancestry. This seems unlikely in mosquitoes, in view of an apparent cyclical activity of the gland cells (*6*).

The presence of an anesthetic component is suggested by observations that the bites of *A. stimulans,* unaccompanied by saliva, are more painful than the bites of normal mosquitoes. Such a component would have considerable adaptive significance and might also be a factor determining the effectiveness of certain species as vectors of disease organisms. The success of insects as vectors has been viewed in this way by Herms (*7*; *8*).

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References and Notes

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8. This study was supported by grants to Professor A. S. West from the Defence Research Board of Canada (6801-19), the National Institute of Allergy and Infectious Diseases (E-1155), and the Ontario Research Foundation.

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Residence Time of Dissolved Phosphate in Natural Waters

Abstract. Residence time varies from approximately 0.05 to 200 hours. Short residence times are indicative of depleted phosphate, active metabolic activity, or both. The turnover rate of phosphate is between 0.1 and 1.0 mg of phosphorus per cubic meter, per hour, regardless of phosphate concentration, except in biologically active systems where it is 1.0 to 20. The turnover rate of phosphate may be more important than the phosphate concentration in maintaining highly productive systems.

The use of $P^{32}O_4$ in studies of lakes has revealed dynamic equilibria between phosphate dissolved in the water and phosphorus in the plankton, benthic organisms, bacteria, sediments, and dissolved organic materials (*1–5*). The residence time of dissolved phosphate proved to be a matter of minutes (*3*). Estimates of the residence time of dissolved phosphate in the sea and in estuaries (Table 1) reveal a wide range of conditions under which phosphate equilibria may be established, with the residence time varying over several orders of magnitude.

Residence time and turnover rate of phosphate in freshly collected water were estimated in the laboratory. Aliquots of 1.5 liters of water were placed in 7-liter rolling bottles in a constant-temperature room, and the water was kept within ±2°C of its temperature at collection. The water was illuminated by flourescent lights with an intensity of 350 ft-ca. Sterile, carrier-free $P^{32}O_4$ was added to the water, and changes in radioactivity were measured at intervals until equilibrium was reached. Uptake of P^{32} by bacteria and plankton was measured by filtering aliquots of water through Millipore filters of 0.45-μ porosity and counting the activity of the dried filters. The residence time of phosphate was calculated by the method used in studies of lakes (*2–4*), but only the observations of Rigler (*3*) are comparable in the method of filtration.

Turnover rate is considerably less variable than residence time, and exceeds the range of 0.1 to 1.0 mg/m³ per hour only in biologically active systems, such as plankton blooms, salt marshes, and small lakes. Residence time is influenced both by the turnover rate and the concentration of dissolved phosphate. Therefore, a system having a short phosphate residence time may be impoverished in phosphate, as in the sea, or it may be unusually active biologically, as in algal blooms. When both conditions occur together, as in small lakes, the residence time becomes vanishingly short.

Measurements of the concentration of dissolved phosphate in natural waters give a very limited indication of phosphate availability. Much or virtually all the phosphorus in the system may be inside living organisms at any given time, yet it may be overturning every hour with the result that there will be a constant supply of phosphate for organisms able to concentrate it from a very dilute solution. Such systems may remain stable biologically and chemically for considerable periods in the apparent absence of available phos-

Table 1. Residence time, concentration, and turnover rate of dissolved phosphate in natural waters

Date	Location	Type of system	Dissolved phosphate			T (°C)
			Res. time (hr)	Concn. (mg atom P/m³)	Turnover (mg P/m³ per hr)	
9/28/54	Oiseau Lake, Ontario*	Small lake	0.06	0.003	1.6	
9/11 and 9/18/53	Toussaint Lake, Ontario*	Small bog lake	0.08	0.009	3.6	
9/17/54	Maskinonge Lake, Ontario*	Lake	0.4	0.012	1.0	
7/29/58	Salt-marsh creek, Georgia	*Kryptoperidinium* bloom	1.0	0.6	19	29
5/14/59	Altamaha River, Georgia	Nostocaceae bloom	1.0	0.1	3	25
7/18/58	30°53′N, 80°28′W	Continental shelf water	5	0.1	0.6	29
7/18/58	30°58′N, 80°01′W	Gulf Stream, surface	4	0.1	0.8	29
7/18/58	30°58′N, 80°01′W	Gulf Stream, 60 m	12	0.1	0.3	29
4/2/59	31°25′N, 81°05′W	Coastal sea water, surface	34	0.1	0.1	18
10/15/59	31°19′N, 81°10′W	Coastal sea water, surface	155	0.5	0.1	34
10/15/59	31°20′N, 81°13′W	Coastal sea water, surface	63	0.8	0.4	33
11/19/59	31°20′N, 81°13′W	Coastal sea water, surface	50	0.3	0.2	15
11/19/59	31°19′N, 81°11′W	Coastal sea water, surface	46	0.1	0.1	16
4/20/59	31°23′N, 81°17′W	Doboy Inlet	4	0.2	1.5	22
11/12/59	31°23′N, 81°17′W	Doboy Inlet	66	1.0	0.5	16
11/19/59	31°23′N, 81°17′W	Doboy Inlet	111	0.9	0.2	15
6/26/58	31°25′N, 81°18′W	Doboy Sound	37			
2/28/59	31°25′N, 81°18′W	Doboy Sound	50	0.6	0.5	12
7/17/59	31°25′N, 81°18′W	Doboy Sound	56	1.0	0.5	30
11/12/59	31°25′N, 81°18′W	Doboy Sound	39	1.0	0.8	15
11/19/59	31°25′N, 81°18′W	Doboy Sound	30	1.0	1.0	14
1/30/59	31°29′N, 81°16′W	Salt marsh (low tide)	49	3.0	2.0	15
10/12/59	31°29′N, 81°16′W	Salt marsh (low tide)	40	5.5	4.0	27
11/12/59	31°29′N, 81°16′W	Salt marsh (high tide)	169	1.1	0.2	15
11/12/59	Altamaha River, Georgia		13	0.2	0.5	14
9/14/54	Ottawa River, Ontario*		30	0.05	0.2	

* From Rigler (*3*). Rigler's observations have been converted from other units of measurement for convenient comparison.