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The Salivary Gland Poison of the Aëdes Aegypti.

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The nature of the poison contained in the salivary glands of mosquitoes is little understood. That this substance is very irritating to the human skin (and to that of other animals) has long been an established fact. Those who dwell in the tropical belt are well aware of this though the nuisance of mosquitoes is by no means limited to warm climates. Not all species of mosquitoes bite man, indeed some seem to prefer animal blood to that of man, but a human blood meal is usually acceptable if no other is available. On the other hand, there are many different kinds of mosquitoes which prefer the blood of man and in some instances refuse to partake of the blood of certain animals. When the mosquito bites, some of the salivary secretion is injected into the wound and this, in most instances, is followed by the appearance of a wheal, surrounded by an area of erythema and accompanied by intense itching. This is the characteristic reaction following the bite of the *Aëdes aegypti* and we have been interested in the substance contained in the salivary glands of this particular insect which causes this reaction.

People vary in susceptibility to this poison. Some state that mosquitoes never bother them. Others develop the most intense reactions following these bites. Still others state that they believe that they have become immune to certain mosquitoes. Several individuals have reported to us that 2 or 3 years ago, when they first came to Porto Rico, they were bothered by mosquito bites and developed intense lesions following them and that after 2 or 3 years in residence here they have gradually become "immune" to mosquitoes and seriously doubt if they are ever now bitten. The evidence seems to justify the opinion that prolonged residence in the presence of mosquitoes and exposure to their bites produces a form of immunity against the specific poison elaborated by these insects. However, we have found that this is not always true. One of our staff who is accustomed to breed the *Aëdes aegypti* in his laboratory and allow these insects to feed frequently from his arm, developed an intense reaction with the poison extracted from the salivary glands of this insect. This test, however, was made after his absence from Porto Rico for 4 months and there is the possibility

that an acquired immunity to this poison does not persist for any length of time. The fact remains that certain individuals become immune to the mosquito salivary gland poison after prolonged contact with the insect and it is upon this observation that this study has been based.

In 1888 Macloskie[1] demonstrated that the mosquito has 2 salivary glands and that each of these glands possesses 3 lobes. He found that the middle lobe of each gland differs from the others in having evenly granulated contents and staining more deeply. He regarded the middle lobe as the source of the poison. Bruck[2] in 1911, extracted a poison from the bodies of mosquitoes with water, glycerine and chloroform, which he called *culicin*. He assumed that this poison came from the salivary gland and found that it produced lesions in his own skin similar to the mosquito bite itself. Furthermore he demonstrated that the poison possessed hemolytic powers. Nuttall and Shipley,[3] working with *Culex pipiens*, extracted the salivary glands from 6 sets (36 acini) with salt solution and found that their extract neither prevented coagulation nor caused hemolysis of human blood. Schaudinn[4] observed certain yeast cells in the oesophageal diverticula (sucking stomach) of the mosquito which he believed were normal and constant commensals of the insect. He further noted that the sucking stomach emptied itself from time to time due to certain convulsive contractions of the abdomen during feeding. Schaudinn was unable to produce any irritating effect by injecting extracts of the salivary glands themselves "under the skin" though he produced noticeable and characteristic itching irritation by introducing the oesophageal diverticula with their content of yeast into an opening in the skin. From these experiments Schaudinn believed that the irritating action of the mosquito is caused by an enzyme from a commensal fungus. Yorke and Macfie[5] have shown that an emulsion of the salivary gland of *A. muclipennis* agglutinates the erythrocytes of human blood but that emulsion of the stomach and ventral oesophageal diverticulum has no such action. These authors found that the salivary gland emulsion of *Culex pipiens, Theobaldia annulata, Stegomyia fasciata* and *Glossina tachninoides* do not agglutinate human red blood cells. No hemolysin was detected in any of the insects studied by them. The salivary gland emulsion from *A. maculipennis* and of *Glossina tach-*

[1] Macloskie, *Am. Naturalist*, 1888, xxii, 884.

[2] Bruck, *Deutsche medizin. Wochenschr*, 1911, 1787.

[3] Nuttall and Shipley, *J. Hyg.*, 1901-3, i, ii, iii.

[4] Schaudinn, *Arb. aus dem kais. Gesundheitsamte*, 1904, xx, 387.

[5] Yorke and Macfie, *Ann. Trop. Med. and Parasitol.*, 1924, xviii, 103.

ninoides possessed a definite anticoagulating substance but this was not true for *Stegomyia fasciata*. More recently Hecht[6] described some interesting experiments dealing with this subject and has collected the literature concerning it.

In our experiments we have been interested in the toxic substance contained in the salivary glands of the *Aëdes aegypti* and in the reaction this substance produces in different individuals. We have carefully dissected out over 2000 salivary glands from female *A. aegypti* bred in the laboratory. Several very potent extracts have been prepared from these glands by grinding the glands with sand in a sterile mortar and extracting with physiological salt solution. One type preparation was made with approximately 1300 glands and extracted with 15 cc. of saline. After filtering through a Berkefeld "N" filter this material was employed for "intradermal" injection in 0.1 cc. amounts. Other similar preparations were made, using various numbers of glands and smaller quantities of saline for the extraction. We have tested this material on a large number of individuals and have obtained results which fall into 3 distinct classes. (1) Those individuals who give a typical, distinct and characteristic reaction which to all appearances is the same as a bite caused by this insect; (2) Those individuals who give no reaction whatever; and (3) those individuals who give a "delayed" reaction, coming on from 8 to 18 hours following the injection. The typical positive reaction appears in from 2 to 5 minutes following the injection and consists of a wheal (¼ to ½ inch in diameter) surrounded by an erythema varying from 1½ inch to 2 inches in breadth and length. The reaction is accompanied by intense itching and persists for approximately one hour. Reactions are not obtained in susceptible individuals if the extract is injected *under* the skin but only if given intradermally. This would readily explain the negative results obtained by Schaudinn. There is a certain agreement, but by no means constant, in our results which suggests that individuals after prolonged stay in association with this mosquito, are immune to this poison. Dark skinned individuals seem to be more immune than those having fair skins. The delayed reaction is difficult to understand and we offer no explanation for this. We have been unable to immunize individuals by repeated injection of the poison; they are still susceptible to the mosquito and to the extract after 8 or 10 daily injections. Furthermore we have been unable to produce local immunity in a given skin area by repeated injections of the extract. Intravenous injection of the ex-

[6] Hecht, *Arch. f. Schiffs u. Tropen-Hygiene*, 1928, xxxii, 561.

tract into rabbits in an attempt to produce an immune serum have failed to produce a serum which would neutralize the poison. The poison is not destroyed by freezing to —12° C. for 5 hours or by heating in a boiling water bath for 10 minutes. The extract does not produce hemolysis of human red cells or does it prevent coagulation of the blood. This is in agreement with the work reported by Yorke and Macfie on this mosquito. The extract is without effect when injected intracutaneously into guinea pigs. The nature of this toxic substance remains unknown (toxin, foreign protein, etc.) but will be the subject for future investigation.

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Phloridzin Diabetes and Vital Staining.

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Since the introduction in experimental pathology of vital staining of animals this method has been widely employed for the study of the physiology of the macrophage (Reticulo-Endothelial) system. In a number of instances this method has been used in conjunction with bacterial infections in the experiments with the so-called blockade of the reticulo-endothelial apparatus.

The investigation to be reported concerns itself with experiments on vital staining of animals treated with phloridzin. This substance leads to an outstanding glycosuria which, according to older observers is renal in origin. Present investigations have shown that beside the kidneys chronic phloridzin intoxication also leads to marked pathologic changes in other visceral and hematopoetic organs.

From a chemical standpoint, although the intimate mechanism of the glycosuria is not clear the loss of the function of carbohydrate oxidation is considered as being almost complete; and for practical purposes the disease is regarded as being close to diabetes mellitus (Nash[1]). It is obvious that the faulty metabolism of the carbohydrates carries with it an impairment in the combustion of fats and proteins.

Chronic phloridzin intoxication is therefore from histopathologic

[1] Nash, T. P., *Phys. Reviews*, 1927, vii, 385.