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THE STRUCTURE AND FUNCTION OF THE APICAL LABRAL PEGS AND LONG LABELLAR HAIRS OF THE MOSQUITO *AEDES AEGYPTI* (L.)

by

© Thomas Reddington Pearson

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

EDMONTON, ALBERTA

FALL, 1970

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled THE STRUCTURE AND FUNCTION OF THE APICAL LABRAL PEGS AND LONG LABELLAR HAIRS OF THE MOSQUITO AEDES AEGYPTI (L.) submitted by Thomas Reddington Pearson in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Supervisor

External Examiner

Date Fall 1970

BIOGRAPHICAL SKETCH

I was born April 25, 1941 in San Jose, California. I graduated from St. Emydius grammar school in 1956 and from Riordan High School in 1959. I received a B.A. degree in zoology from San Francisco State College in 1963. I was employed from 1962-1966 at the University of California Medical Center at San Francisco, Department of Dermatology. My research at this time was centered around the behavioral responses of mosquitoes in relation to host attraction and repellancy. Upon completion of my Ph.D. it is my desire to obtain a teaching-research position at the university level.

ACKNOWLEDGEMENTS

I am greatly indebted to Drs. B. Hocking, D. A. Craig, and R. H. Gooding for their continued interest and advice. I would also like to thank Drs. R. B. Stein and A. French of the Department of Physiology for their constant help and the use of their laboratory. Thanks are also due to my fellow graduate students especially M. A. C. Chance, A. Holden, and P. Chiang for their help and advice.

Finally I would like to thank my wife Susan Pearson and my parents Mr. and Mrs. R. W. Pearson for their help and encouragement.

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ABSTRACT

The external structure of the apical labral pegs and long labellar hairs of Aedes aegypti (L.) was studied with the scanning electron microscope. In addition stained transverse sections of these structures were made and studied under the light microscope in an attempt to trace their innervation. The labral nerves were followed from the proximal origin at the frontal nerve to the apical pegs. The labial nerves were followed to the labellar lobes. No individual nerve cell bodies could be associated with either type of organ.

Both the labral pegs and the long labellar hairs were studied electrophysiologically with chemical and mechanical stimuli. No response was found in either organ after chemical stimulation but the long labellar hairs were found to respond to both step deflections and time-varying deflections. The labral pegs do not respond to mechanical stimuli. The addition of random noise was found to overcome distortions due to rectification at low frequencies and phase locking at high frequencies, and extended the low frequency range of sensitivity.

No response of the labral pegs resulted from either chemical or mechanical stimuli. The long labellar hairs do not respond to chemical stimuli but do respond to mechanical stimuli. Because of the above conclusions it is also

concluded that primary food detection may take place in the cibarium.

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I. INTRODUCTION

The role of contact chemoreceptors in the detection of food by mosquitoes has been demonstrated with behavioral techniques by Frings and Hamrum (1950), Owen (1961) and Hosoi (1959).

More recently behavioral studies by Owen (1963) have provided evidence for the presence of taste receptors on the tarsi, labella, ligula, and within the cibarium. Direct stimulation of the chemosensory hairs of the tarsi, labella, and ligula with water and various sugars results in characteristic prefeeding and feeding movements of the head and mouthparts. Stimulation by blood results in no feeding behavior. Owen concludes that the factors which attract a mosquito to a host also elicit initial feeding behavior. He attributes a sustained feeding response to subsequent stimulation of the cibarial receptors.

The structure of the labral apical organs has been previously studied with the light microscope (Vogel, 1921; Robinson, 1939; and von Gernet and Buerger, 1966). Hosoi (1954), working with the food distribution system of mosquitoes, found that the sensitivity to blood decreased slightly when the tip of the labrum was excised. Hosoi therefore concluded that the sensilla on the tip of the labrum may play an initial role in the identification of blood. Since the sensitivity to blood merely decreased after labral excision probably other sensilla also enable

mosquitoes to recognize food. In the same study Hosoi showed that the labrum was insensitive to glucose. Stimulation of the labrum alone with erythrocytes led to gorging, whereas stimulation of the labella with erythrocytes produced negligible results.

It was my purpose to carry out the following: 1) a detailed study of the external and internal structure of the apical labral pegs and the labellar hairs of Aedes aegypti (L.). An attempt was made to trace the innervation to these organs to establish the number of specific receptor cells involved with each organ. 2) A study of the electrophysiological response of the labral apical pegs and long labellar hairs to both chemical and mechanical stimuli.

The suspicion that these organs may be important in both primary food detection and specific food identification including shunting of blood to the midgut and sugars to the oesophageal diverticula make an understanding of the structural-functional relationship justifiable and essential.

II. MATERIALS AND METHODS

1. Biological Material:

Aedes aegypti (L.) female mosquitoes were used exclusively for the morphological and electrophysiological studies. The colony has been reared for several years in this laboratory and has probably been mixed with other strains and can therefore be considered a wild strain. The adults were reared from stored eggs in the room where the experiments were performed.

Ground up Purina® rat pellets were used for larval food. The larvae were reared in enamel pans with a density not exceeding 250 larvae/liter of water. The larval medium was kept clear of fungus and kept at a constant level. Emerging adults were split into two groups, one for experimental use and one for breeding purposes only. The breeding colony was maintained in a 1 ft^3 cage on 5% sucrose solution with weekly blood meals. The experimental colony was kept in separate cages, 6" x 6" x 12", according to age and fed 5% sucrose solution until 24 hrs. prior to testing. During the 24-hr. pre-test period the mosquitoes were fed distilled water only. The test mosquitoes were fertilized and between 3 and 8 days old.

2. Morphological Studies:

2.1 Light Microscope Studies:

2.11 Methods:

Fifty adult female Aedes aegypti (L.) were prepared for observation with the light microscope. The mosquitoes used were between 3 and 8 days old.

Either whole live mosquitoes or heads with intact mouthparts from freshly killed mosquitoes were fixed for at least two days in Bouins solution. After fixation the tissue was dehydrated to 99% ethanol in the usual manner and then double embedded according to the method of Peterfi.

Transverse sections 4 and 10μ thick of the labrum and labium were cut using a rotary microtome. The sections were stained either with hematoxylin and eosine or Gomori's chrome hematoxylin-phloxine (C.H.P.) and mounted in Canada balsam.

Whole mounts of the labella and labrum were made but I decided that the scanning electron microscope provides a more efficient method for studying the external morphology of these structures.

2.2 Scanning Electron Microscope Studies:

2.21 Methods:

The heads of female mosquitoes were either air dried or freeze dried. Both methods of drying proved to be successful with the freeze drying process being somewhat faster. The material was mounted and orientated on a piece of double stick cellophane tape which was attached to the mounting stub. Several sets of mouthparts can be mounted

with different orientation on a single stub. The final step prior to viewing was to vacuum coat the tissue with a thin film of gold/palladium. The prepared material was viewed and photographed on either a Jeolco model JSM-U3 microscope or a Cambridge Stereoscan II microscope.

3. Electrophysiological Studies:

3.1 Chemical Stimulation:

3.11 Materials:

The following chemicals were all obtained from Sigma Chemical Co.: adenosine-5'-triphosphate (barium salt), NaCl, $MgCl_2$, KCl, sucrose, and D-glucose. $CaCl_2$ and LiCl were obtained from Fisher Scientific Co. Choline chloride was obtained from Eastman Kodak. A sample of adenosine-5'-triphosphate (lithium salt) was obtained from Nutritional Biochemical Co. All of the chemicals used were the analytical grade supplied by the manufacturer. The chemicals used in repeated trials were always obtained from the same bottle in order to minimize the introduction of different impurities to the test solution.

3.12 Methods:

All test solutions were made up using demineralized glass distilled water. The solutions were initially made up in either 1 molar or 2 molar stock solutions. Further dilutions were then made up from the stock solutions and

the pH measured and recorded. Because I wanted to test the chemicals in as pure a form as possible no effort was made to adjust the pH of the solutions; however the range of pH for given concentrations from different stock solutions remained very constant. The solutions were at all times sealed in glass containers and stored in a refrigerator at 34 F until just prior to testing.

The test chemicals were applied in the same way to both the tip of the labrum and to the labellar hairs. The test solution was drawn into a 2 ml syring fitted to a 25 gauge hypodermic needle. The solution was then injected into a glass micro-capillary tube which was heat-drawn to a tip diameter of 20μ. The filled capillary tube was then placed in a test tube containing a reservoir of the test solution. Each test tube was then placed at an angle of about 20° to vertical so that the reservoir just covered the capillary tube preventing evaporation yet permitting easy access to each. With this method several capillaries could be filled with a series of solutions and kept ready for use during an experiment. When in use the capillaries were attached with plasticine to a Leitz micromanipulator. Stimulation was effected by bringing the lumen of the tapered tip of the capillary in contact with either a single hair or the tip of the labrum. I found that with this method smooth contact between the test liquid and hair could be made without mechanically bending the hair. The hair projected inside the capillary, allowing me to easily

bend the hair and stimulate it with a chemical simultaneously. Evaporation from the capillary tip rapidly concentrates the simulating solution (Wolbarsht and Dethier, 1958; Evans and Mellon, 1962). I circumvented this problem by placing a piece of clean filter paper in contact with the solution at the tip of the capillary resulting in a small amount of the solution being drawn out. This procedure was repeated immediately before placing the capillary over the hair. All solutions were tested at room temperature which was kept constant between 20 and 22 C. The solutions were applied in ascending order of concentration to avoid saturating the receptor site. I thought that by doing so the threshold concentration could be determined. The ranges of concentrations used for the various chemicals were based on values found in the literature determined from behavioral studies on various insects. Table 1 lists these ranges along with the behavioral thesholds and references. Precise rejection thresholds for Diptera for KCl, LiCl , and $CaCl_2$ could not be found in the literature. Frings (1948), however, lists rejection values for the caterpillar Eacles imprialis Drury and these values are included in the table and were used as a guide for this work. Test solutions were applied to the sensilla for varying lengths of time, (10 sec - 1 min). A recovery period of 3 min was allowed between tests. At the beginning of this study the method of combining the recording electrode with a stimulating capillary containing the test

Table 1.: Chemical stimulants used in this study with established response thresholds for insects.

STIMULI	RANGE OF CONCENTRATIONS	THRESHOLD	INSECT	REFERENCE
Sucrose	.005 M - 2 M	0.0107 M*	Culiseta inornata	Feir et al., (1961)
D-Glucose	.01 M - 2 M	0.425 M**	Culiseta inornata	Feir et al., (1961)
NaCl	.05 M - 1 M	0.1 M**	Phormia regina	Gillary (1966)
KCl	.05 M - 2 M	0.6 M**	Eacles impirialis	Frings (1948)
$CaCl_2$	.01 M - 2 M	1.78 M**	Eacles impirialis	Frings (1948)
LiCl	.01 M - 2 M	1.80 M**	Eacles impirialis	Frings (1948)
ATP	10^{-2} M	10^{-3} M*	Aedes aegypti	Galun et al. (1963)

* Acceptance Threshold
** Rejection Threshold

chemical was used (Hodgson and Roeder, 1956). The method consists of filling the pipette with a combination of the test chemical and 0.001 M NaCl. An Ag-AgCl wire is inserted into the barrel of the capillary which acts as the recording electrode. The 0.001 M NaCl is below the threshold of all insect chemoreceptors studied so far (Hodgson and Roeder, 1956) and therefore is probably non-stimulating to the mosquito.

3.2 Mechanical Stimulation:

3.21 Methods:

The labellar hairs were stimulated with both time-varying and constant deflection. In addition the hairs were subjected to both constant mechanical deflection and chemical stimuli simultaneously. The tip of the labrum was also stimulated mechanically by means of a constant deflection stimulus.

The prolonged mechanical deflection of the receptor maintaining a steady displacement was effected by bringing a glass capillary in contact with the tip and moving the capillary with a Leitz micromanipulator. No attempt was made to rigidly standardize the magnitude of deflection. However, measurements made during deflection indicated a maximum deflection of 0.1 mm. I determined experimentally that very minute deflections and deflections of around 0.1 mm produced identical responses. I therefore concluded that this variation in stimulation was not a factor which would

significantly affect the response.

The labellar hairs were subjected to time-varying mechanical deflections of known amplitude, frequency, and shape. A wave form generator, Hewlett Packard model 3300A was used as the source providing sinusoidal, triangle, or square wave forms of controlled amplitude and frequency. The signal from the generator was led via an operational amplifier to a Pye-Ling vibration generator (model V47) which transformed the electrical pulse into a very accurate mechanical representation. The mechanical deflection was monitored with a Hewlett Packard displacement transducer (Series 7DCDT) and a negative feedback circuit was employed to reduce mechanical distortion. A glass capillary was attached to the mechanical output of the vibration generator and was brought to close proximity of the hair with a micromanipulator. Final placement of the capillary was achieved with a DC offset control unit. The amplitude of the mechanical deflection was calibrated and could be controlled with the operational amplifier to give accurately reproducible displacements.

A separate circuit which provided a source for white (Gaussian) noise at various levels was mixed with the input from the wave form generator to determine the effect of random noise plus the analog on the response.

Time-varying stimuli with and without the addition of noise were applied to the hair at frequencies from 1 Hz to 50 Hz. The amplitude of the stimuli was varied between 0.01

mm and 0.1 mm deflection. The duration of the stimulation was 30 sec and a 1 min recovery period was allowed between tests. The above system was also used later for prolonged step deflections.

3.3 Recording Methods:

Female Aedes aegypti (L.) were lightly anesthesized with CO_2 and fixed with masking tape to a plexiglass mounting block which positioned the mouthparts in the horizontal plane. An effort was made to maintain the insect without removal of any appendages, to ensure measurement from the insect under close to normal physiological conditions. The mosquito was allowed to recover from the anesthetic and handling for 20 min and then positioned on the stage of a Leitz MK IV dissecting microscope. The light source was a flexible fiber optics system producing high intensity illumination with minimum heat output. The temperature rise measured at the preparation in air with the light at full intensity was 0.5 C in 10 min. However, the light was used at minimum intensity and reflected from below during tests, producing no significant temperature rise.

The indifferent electrode was a platinum wire placed into the clypeal dome of the mosquito (Fig. 1). The recording electrode was an electrolytically etched tungsten wire etched to a tip diameter of about 1.5μ and uninsulated. The recording electrode was placed in the labium near the

left labial nerve approximately 50μ from the junction of the labium and the labella, when recording from the labellar hairs. An effort was made to visually place the electrode at a constant depth and position whenever comparisons were made. I found that reproducible records from single units could be made with this method. When recording from the labrum the recording electrode was placed in the labrum near the base. The electrodes were mounted on Leitz micromanipulators for the insertion. A "good" preparation was signaled by a drop in the noise level following electrode insertion. The whole apparatus was mounted on a 1" thick steel plate imbedded in a pedestal filled with sand. A combination recording stimulating electrode first described by Hodgson and Roeder (1956) was also employed with the labral and labellar hair preparation in the initial stages of this study but was abandoned because of lack of reliability, (see IV).

The electrode leads went to either a Tektronix type 122 pre-amplifier or an Isleworth model A101 pre-amplifier both having an input impedance of 10 megohms and 0.01μf input blocking capacitors. The input signal was filtered between 100 and 1000 Hz. All amplified input signals were first displayed on a Tektronix type 549 storage oscilloscope for viewing and then led to a Tektronix type 502 oscilloscope for photographing with a Grass kymograph camera model C4. All amplified input signals were also led to an audio amplifier and speaker and to one channel of a

Thermionics T 3000 FM 4 channel instrumentation tape recorder (Fig. 1). During prolonged mechanical stimulation a DC signal was led to another channel of the recorder signaling the approximate onset of stimulation. During time-varying mechanical deflections the signal to the vibration generator, the signal from the length transducer and a square pulse of the same frequency were each led to separate channels of the recorder along with the nerve signal. The recorded signals were then played back and photographed after the experiment.

The responses to time-varying stimuli and step inputs were all analysed with a Digital Equipment Co. Lab-8 computer system. The action potentials were led to a pulse height analyzer which acted as a Schmidt trigger in order to trigger one input of the computer. Usually, where a sinusoidal deflection was used a square pulse synchronized in amplitude and frequency with the sine wave was used to trigger the computer through a second input channel. With this procedure post-stimulus time histograms (French and Stein, 1970) were constructed in the following manner for the frequency ranges used. The storage areas of the computer (bins) were set at a constant number and the bin width was adjusted, depending on the sine input frequency, so that the total number of bins equaled the time of one cycle. Since each bin represents a storage area in time, the number of events (action potentials) occurring at a point in time corresponding to a point on the sine stimulus

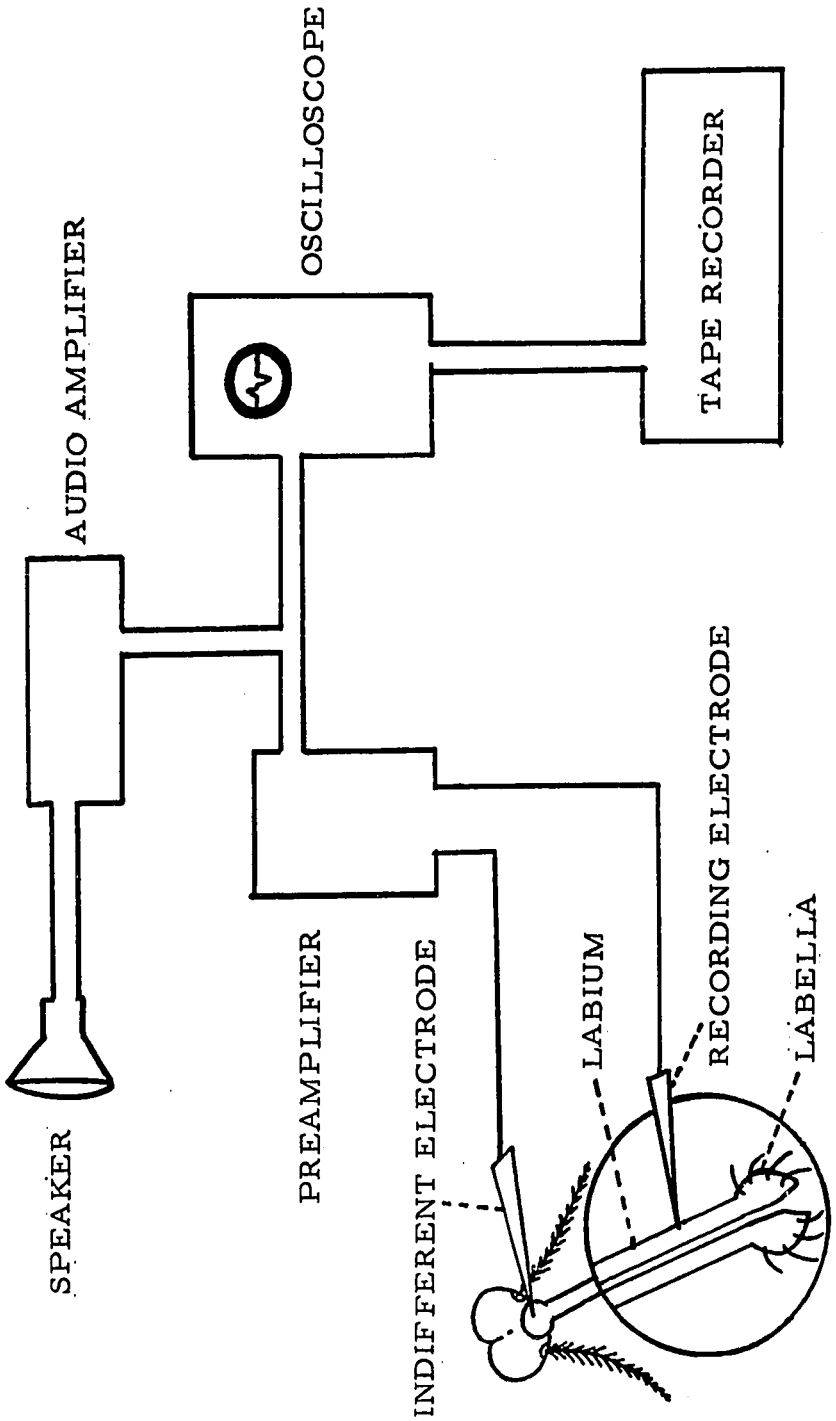


Fig. 1 Electrophysiological recording setup

could be registered and accumulated in the individual bins by superimposing several complete sine cycles. A program designed to plot the best fitting sine curve to the data points in the sense of minimum square deviation from the data points on the histogram and a program for fitting a single exponential to the data points from a square input were used to analyze the data (French and Stein, 1970).

All experiments were performed at room temperature and room relative humidity which were controlled at between 20-22 C and 50-60% RH. Temperature and RH were continuously monitored at the preparation and found to stay within the above ranges.

III. RESULTS

1. Morphology:

To draw conclusions about the function of a receptor system it is necessary to know something about the anatomy of the nervous system in relation to the anatomy of the receptor organ as a whole.

I am assuming that if I place an electrode in close proximity to a receptor that each nerve fiber associated directly with the receptor will respond uniquely to its particular spectrum of stimuli. In other words each nerve fiber will be distinguished by either frequency or amplitude or both. This does not mean that the amplitude will remain constant from preparation to preparation but that the relative differences will persist, making it possible to distinguish responses of each fiber from preparation to preparation.

Because the innervation within the labrum and labium of mosquitoes is largely unknown I studied both the external and internal structure of the receptors and traced the innervation to the receptors.

In the blowfly Phormia regina there is morphological evidence for five neurons in a single labial hair sensillum (Dethier, 1962). Behavioral evidence (Dethier and Evans, 1961), and electrophysiological evidence (Evans and Mellon, 1962), confirm a specific response function for four of the above five neurons. It is thus a great value to know

the number of neurons associated with each receptor and the overall structure of the receptor itself when interpreting physiological evidence. Information about the innervation patterns of the receptors of female A. aegypti follows.

1.1 Labrum:

The labrum along with the other mouthparts of Culicidae are elongate and function as sucking mouthparts. The stylets; labrum, maxillae, mandibles, and hypopharynx lie within a canal in the labium which has an anterior groove. The labrum is further modified by having a posteriorly opening canal extending the entire length of the stylet. This canal acts as the food canal and opens proximally into the cibarium.

The first description of the labral receptors and the innervation to these receptors came from Vogel (1921), working with Culex pipiens L., Anopheles maculipennis Meigen, and Anopheles claviger (Meigen). He described two ventrolateral chitin canals containing long thin filaments terminating distally in the tip in a group of cells associated with fine chitin spines. He further assumed that these spines are sensory in function. Robinson (1939) also described a pair of pegs near the tip of the labrum in Anopheles maculipennis which he thought to be sensory. The work of von Gernet and Buerger (1966) confirms the existence of structures on the labrum which may function as sense organs. They, however, point out that the structures

described by Vogel and Robinson are in fact two sets of structures: 1) an apical set of four pegs divided into medial and lateral pairs located at the tip, and 2) a subapical pair of small pegs set in sockets and located laterally and proximally. Of the 24 species studied by von Gernet and Buerger all the females except those of one species studied of the non-blood sucking genus Toxorhynchites had apical pegs. Hudson (1970), however, demonstrates the presence of apical pegs on Wyeomyia smithii, a species never observed to blood feed on any animal. The males of none of these species had apical pegs, but the presence or absence of subapical pegs bore no relation to sex.

The following two subsections present a further examination of the morphology of the labrum of Aedes aegypti.

1.11 Light Microscopy:

Fig. 2, 3, 4 and 5 show transverse sections selected from a series starting proximally and ending in the tip. In these sections all of the mouthparts are present. The orientation in these plates is anterior side up and the stain is C.H.P.. Fig. 2 shows what the labrum looks like throughout most of its length. The two ventrolateral canals can be seen and contain a branch of labral nerve II. The paired labral nerve is a branch of the frontal connective which connects the suboesophageal ganglion to

the frontal ganglion. About 250μ from the tip the labrum becomes flattened dorso-ventrally and the canals and nerves can still be seen (Fig. 3). In Fig. 4, around 100μ from the tip, the labrum has decreased in size laterally and become more flattened dorso-ventrally. At this point each nerve has split into two smaller processes. Fig. 5 is a section through the apical pegs. The two lateral pegs terminate before the two medial pegs; therefore no definite lumen or cytoplasmic processes can be seen. The medial pegs, however, appear to have a lumen containing an extension of the split nerve in Fig. 4. At no point in the above series was any lateral branching seen leading to the region of the subapical structures.

1.12 Scanning Electron Microscopy:

Fig. 6 shows an electron micrograph of the ventral surface of the labrum about halfway between the base and tip. The food canal can be seen and the ventral opening is discontinuous due to overlapping of the ventrolateral ridges at the right of the picture. This overlapping is not seen in the transverse sections of the above section and is most likely an artifact. Fig. 2 shows the posterior edges of the food canal as separated with the hypopharynx forming the posterior closure of the food canal. The ventrolateral thickenings, which contain the labral nerves, can also be seen in this figure. Fig. 7 shows an electron micrograph of the tip of the labrum from the left ventral

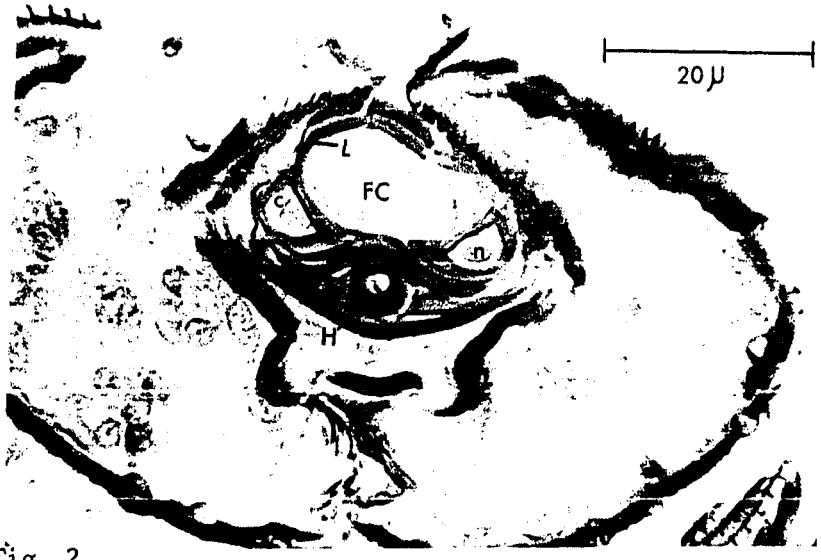


Fig. 2
Cross section through the mouthparts of A. aegypti (L.).
C ventrolateral canal, FC food canal, H hypopharynx,
L labrum, N nerve.

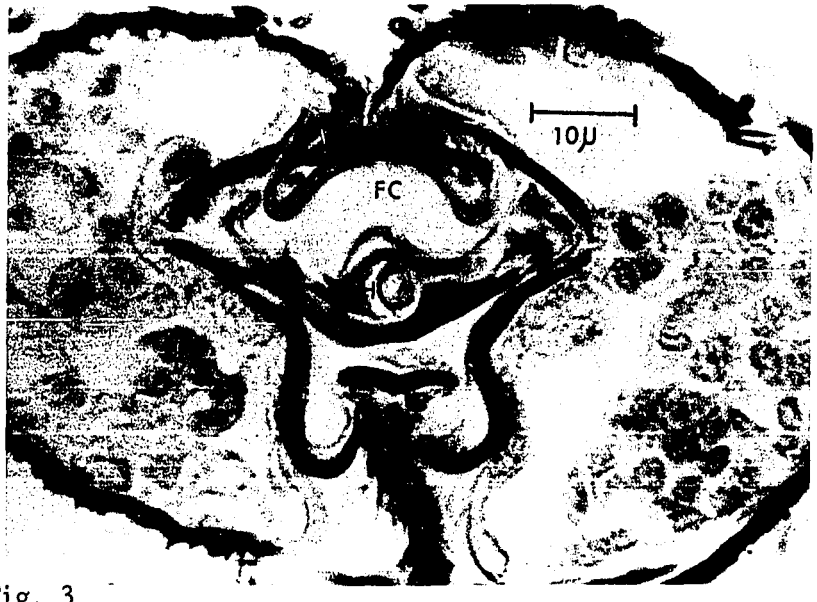


Fig. 3
Cross section through the mouthparts of A. aegypti (L.).
FC food canal, H hypopharynx, L labrum, N nerve.

Fig. 2
Cross section through the mouthparts of A. aegypti (L.).
C ventrolateral canal, FC food canal, H hypopharynx,
L labrum, N nerve.

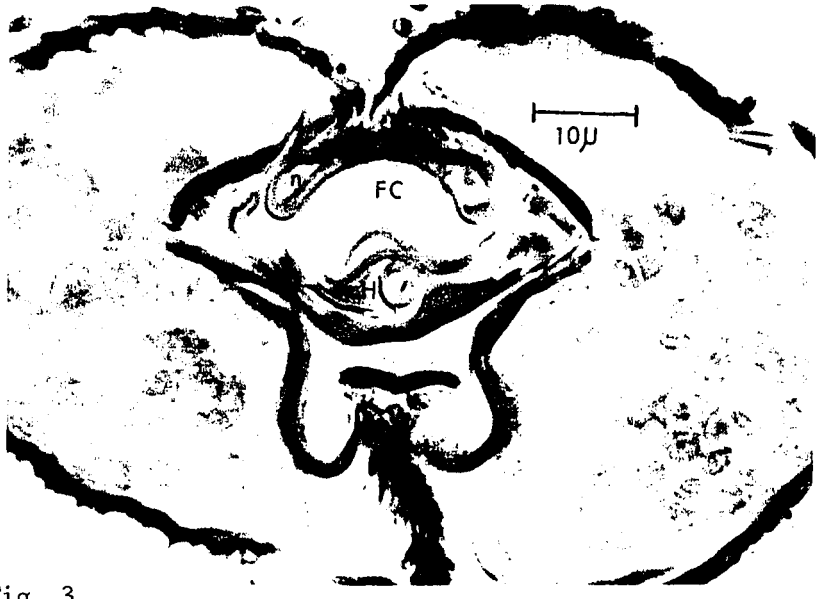


Fig. 3
Cross section through the mouthparts of A. aegypti (L.).
FC food canal, H hypopharynx, L labrum, N nerve.

Fig. 4
Cross section through the mouthparts of A. aegypti (L.) near the tip. C ventrolateral canal, H hypopharynx, L labrum, N nerve.

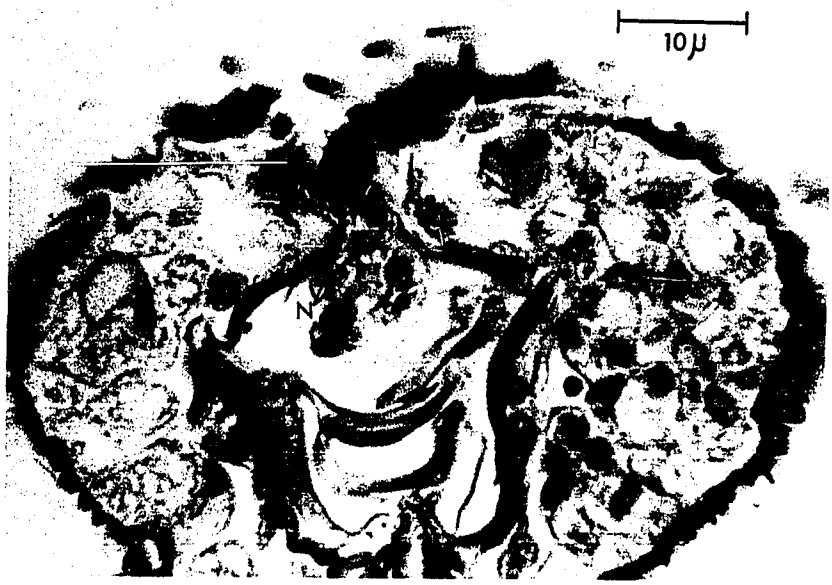


Fig. 5
Cross section through the mouthparts of A. aegypti (L.) at the extreme tip. L labrum, N nerves.

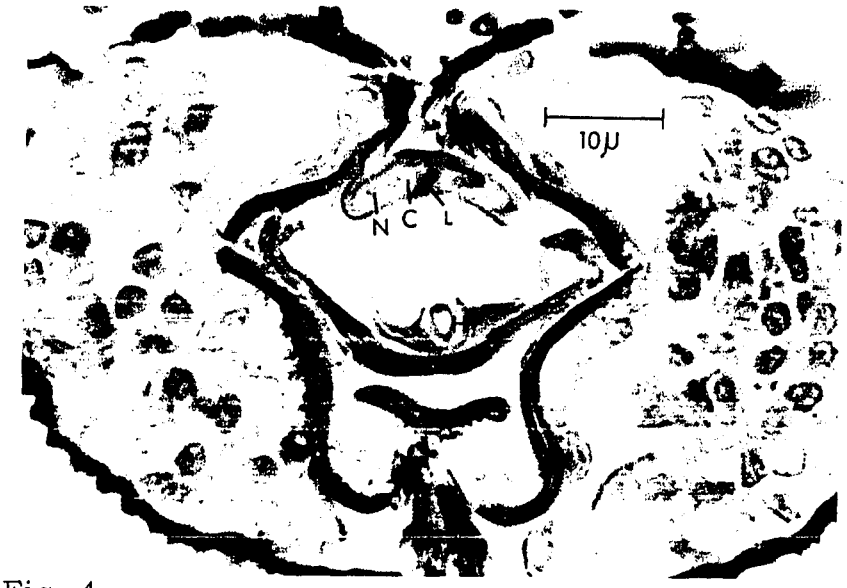


Fig. 4
Cross section through the mouthparts of A. aegypti (L.) near the tip. C ventrolateral canal, H hypopharynx, L labrum, N nerve.

Fig. 5
Cross section through the mouthparts of A. aegypti (L.) at the extreme tip. L labrum, N nerves.

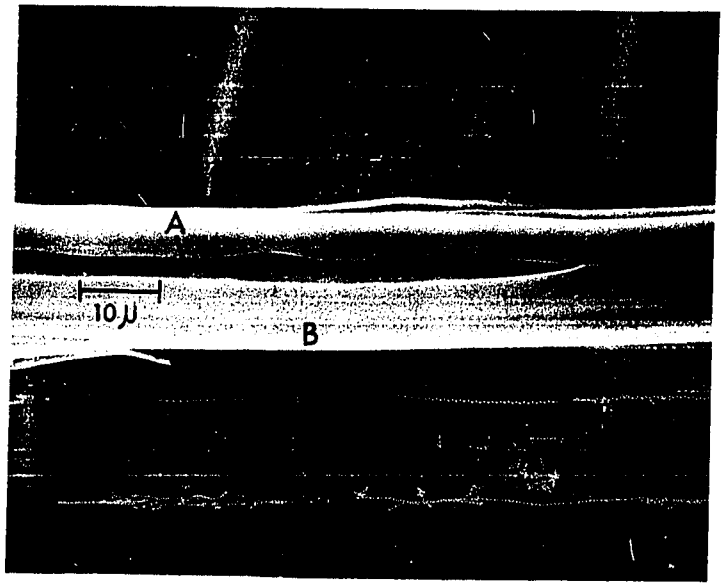


Fig. 6
Scanning electron micrograph of a labrum of A. aegypti (L.). A food canal, B ventrolateral ridges containing canals and nerves. Left ventrolateral aspect.

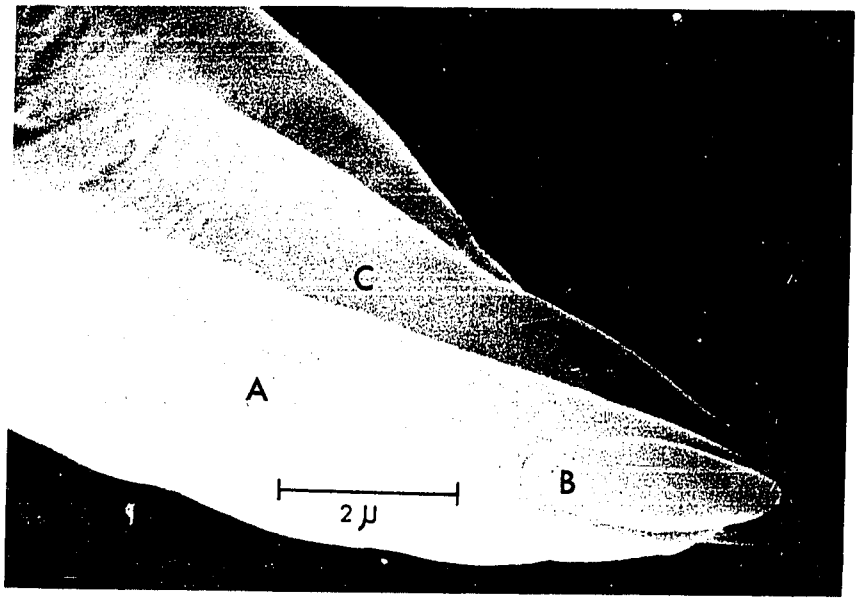
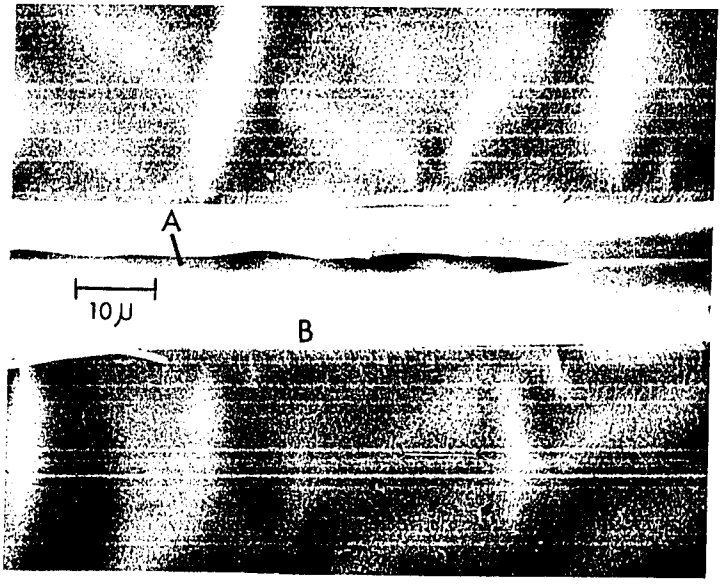


Fig. 7
Scanning electron micrograph of the tip of the labrum of A. aegypti (L.). A lateral pegs, B medial pegs, C beginning of the food canal. Left ventrolateral aspect.



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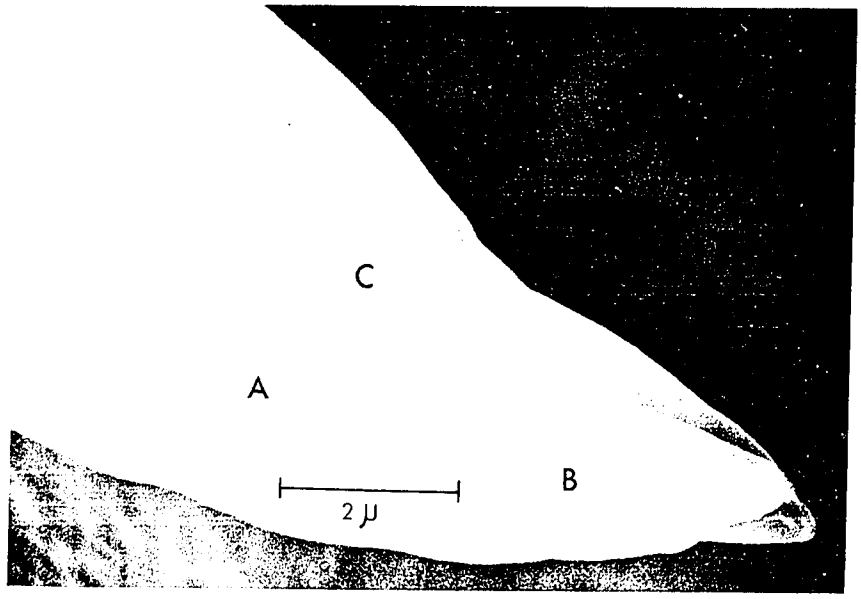

Fig. 7
Scanning electron micrograph of the tip of the labrum of A. aegypti (L.). A lateral pegs, B medial pegs, C beginning of the food canal. Left ventrolateral aspect.

aspect. The apical structures can be seen and are set in sockets. Both the medial and lateral pegs are basiconic, the lateral pegs 2.5μ in length and the medial pegs 3.0μ in length. The pegs are less than 1.0μ in maximum diameter. Slifer, Prestage and Beams (1959) have described very small pores in the cuticle of sensilla basiconica of grasshoppers. No such pores are visible on the labral pegs but this may be due to clogging of the pores because of vacuum coating the tissue prior to viewing, or because of the resolution of the instrument which is 180 Å. The presence of pores as a likely prerequisite for chemoreceptors would indicate that the pegs function as chemoreceptors (Slifer, 1962); the pores presumably linking the interior of the receptor with the external environment.

1.2 Labellar Hairs and Labial Innervation:

In mosquitoes the labium is modified into an elongate structure containing the rest of the mouthparts within an anterior groove. The tip of the labium is modified into the labella, consisting of two independently articulating lobes.

1.21 Scanning Electron Microscopy:

The hairs on the labella can be divided into two major groups based on length: long 40μ hairs scattered over the surface of the lobes, and smaller 10-20μ hairs concentrated at the tip of the lobes (Fig. 8). A similar grouping of hairs on Culiseta inornata has been made by Owen (1963)

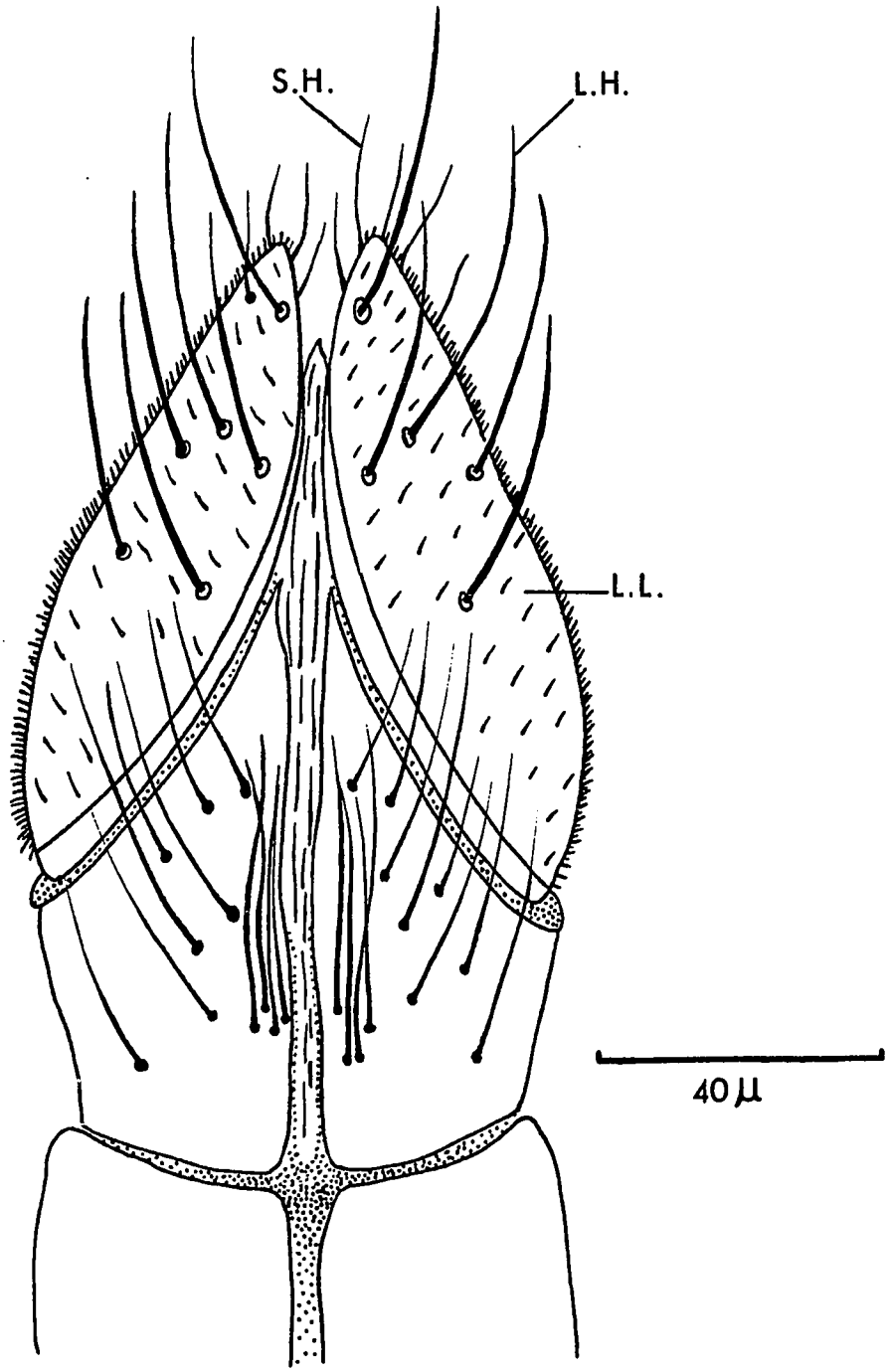

Fig. 8 Drawing of the tip of the labium of A. aegypti showing the labellar hairs. Anterior view. L.L. - labellar lobes, L.H. - long hair, S.H. - short hair.

with a long type 60-80μ and a shorter 32-40μ type. In this study with A. aegypti the longer hairs were worked on exclusively because behavioral studies by Owen (1963) have determined the longer hairs of C. inornata to be chemosensory.

All the labellar hairs are socketed, taper slightly, and curve slightly (Fig. 9A & B). Two types of surface pattern were observed: 1) a chevron pattern as seen in Fig. 9B, and 2) a series of troughs and ridges running parallel with the long axis from the base to the tip (Fig. 9A & C). The chevron pattern has only been seen on one preparation and may be an artifact. The longitudinal pattern has been shown to be present on certain sensilla on the antennae of mosquitoes (Slifer, 1962). Such a pattern would undoubtedly strengthen the hair but this configuration suggests that these hairs are not thin walled. In cross section these hairs would appear as 5-pointed stars with rounded points. Fig. 9C shows the tip of one of these hairs. The most striking feature in this micrograph is the extreme tip which is notched and probably the permeable area of the hair and therefore the only area where chemicals can be detected by the receptor (Dethier, 1962). Owen (1963) reported that the long labellar hairs of C. inornata terminated in a dark staining papilla, the papilla being the permeable area.

1.22 Light microscopy:

Serial transverse sections of the labella and labium

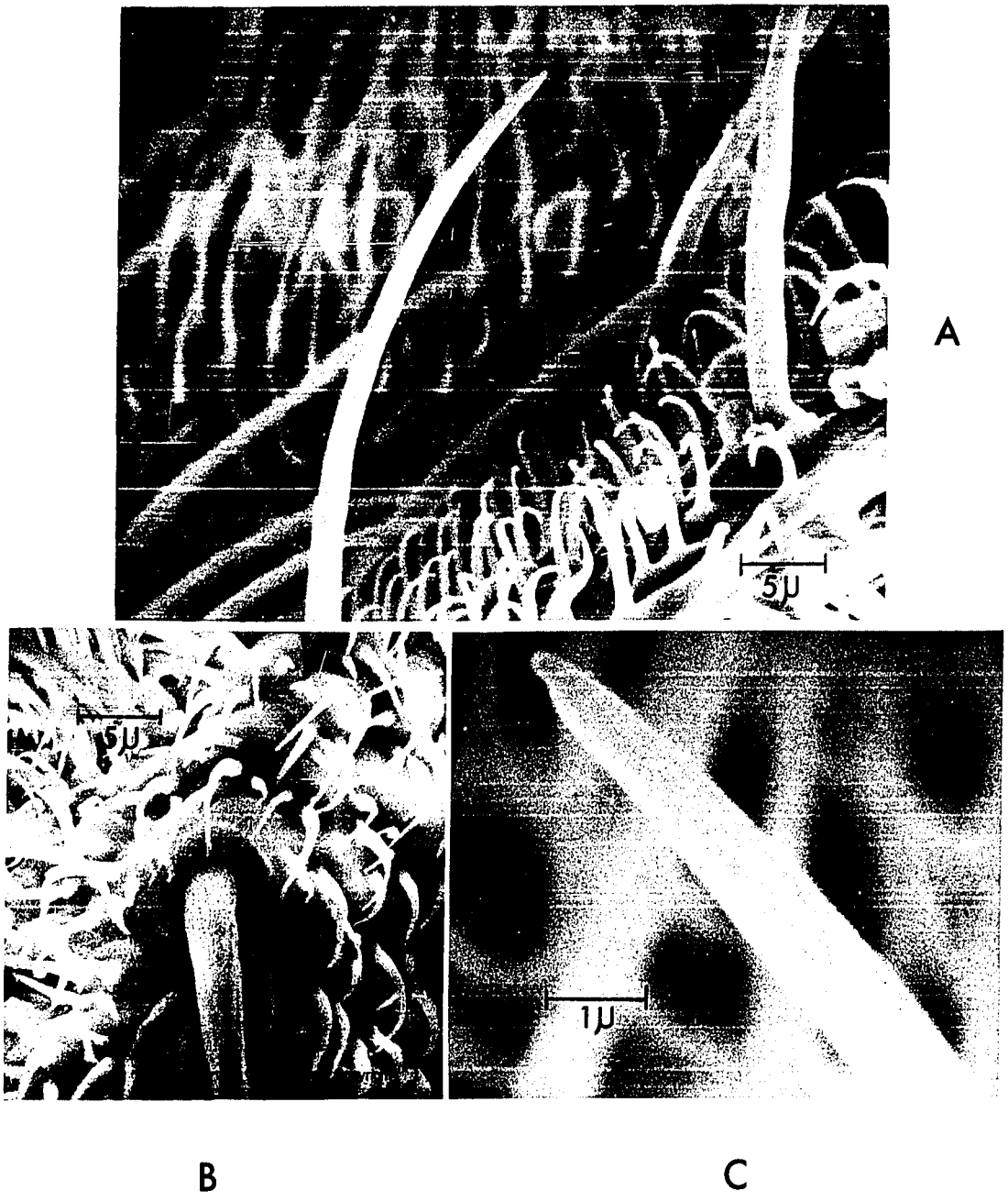

Fig. 9 Scanning electron micrograph of a long labellar hair of A. aegypti. A - 3000 X, B - 2500 X, C - 15,000 X.

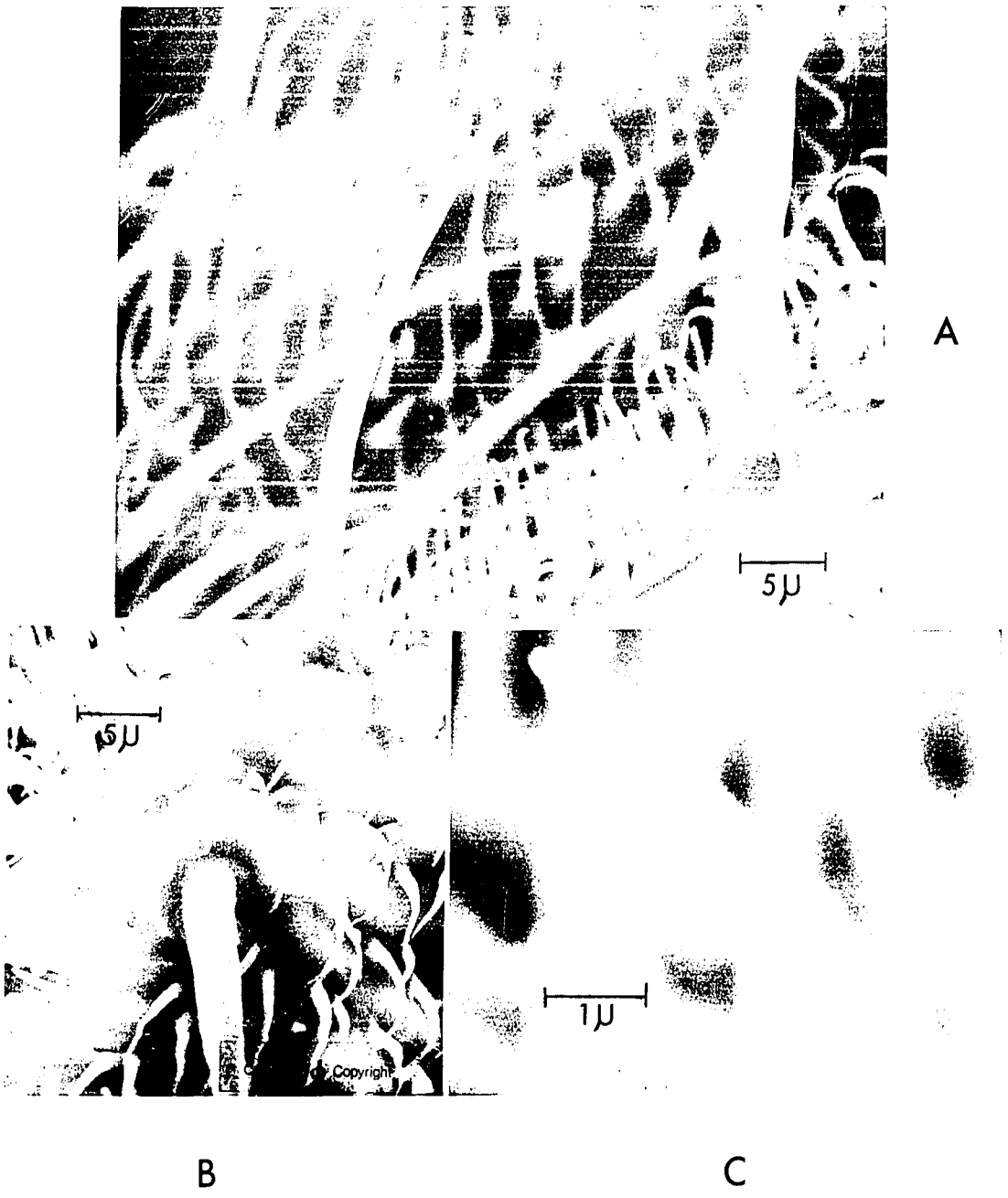

Fig. 9 Scanning electron micrograph of a long labellar hair of A. aegypti. A - 3000 X, B - 2500 X, C - 15, 000 X.

were made to determine the general pattern of innervation and the specific receptor innervation. Fig. 10 shows a section midway along the extension of the labium, muscle bundles and two lateral nerve trunks can be seen. The nerves have been followed proximally to the suboesophageal ganglion as their point of origin. The nerves are compound containing sensory and probably motor neurons, the latter leading to the muscle bundles. Although no motor neuron connections to the labial muscles have been seen no other nerves have been identified in the area making these the only source of motor neurons. The muscles originate near the base of the labium and insert distally at the point where the labellum articulates with the rest of the labium. The function of these muscles is to spread and close the labella and possibly aid in raising and lowering of the entire labium. All of these activities are important in this study because they are the behavioral criteria established for acceptance and rejection by mosquitoes of contact stimulants (Frings and Hamrum, 1950). Fig. 11 shows a transverse section through the labium with a lateral process leading from the labial nerve to a socketed hair. Only the base of the hair is visible in this section. I have seen no reports of sensory structures along the length of the labium in mosquitoes but this section clearly proves their existence. Sections through the labella present a very confusing picture. Hairs with deep sockets resembling those formed from the tormogen

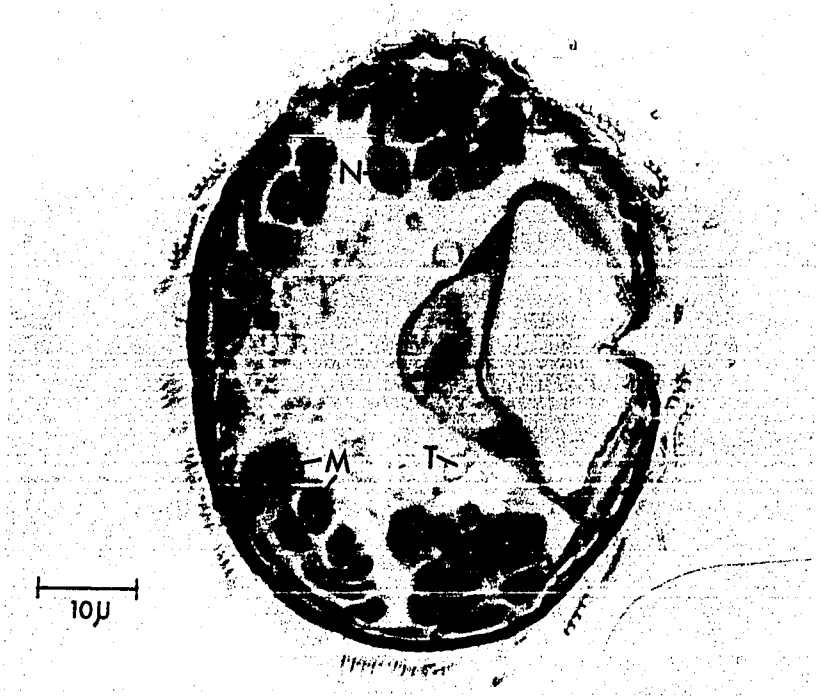

Fig. 10
Cross section through the labium of A. aegypti (L.). M muscle, N labial nerve, T trachea.

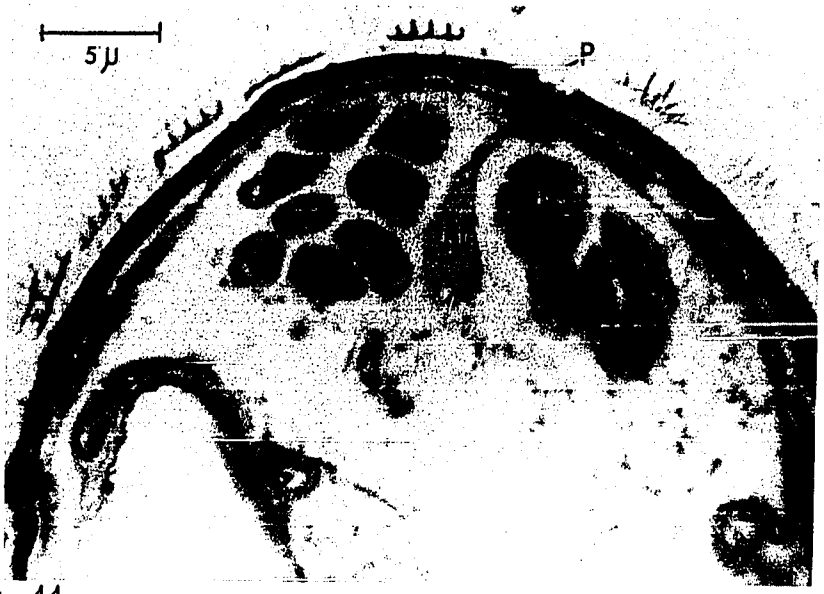

Fig. 11
Cross section through the labium of A. aegypti (L.) showing a lateral branch of the labial nerve to a sensory peg. M muscle, N labial nerve, P sensory peg.

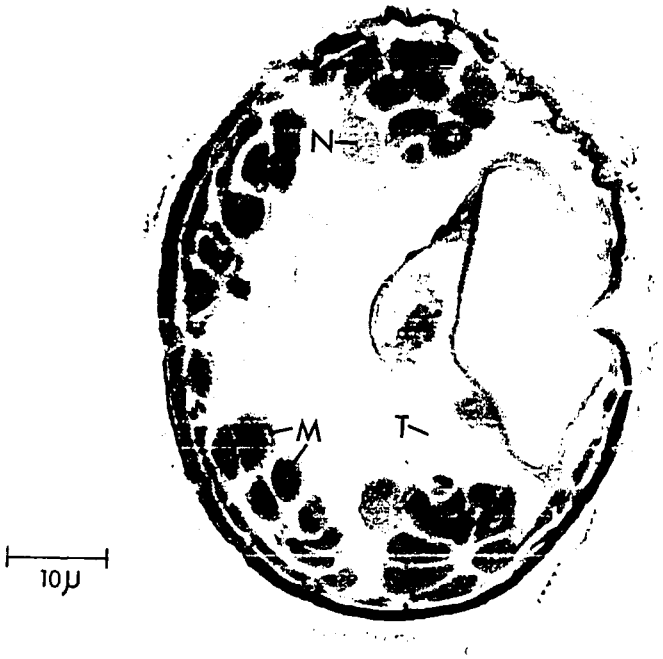

Fig. 10
Cross section through the labium of A. aegypti (L.).
M muscle, N labial nerve, T trachea.

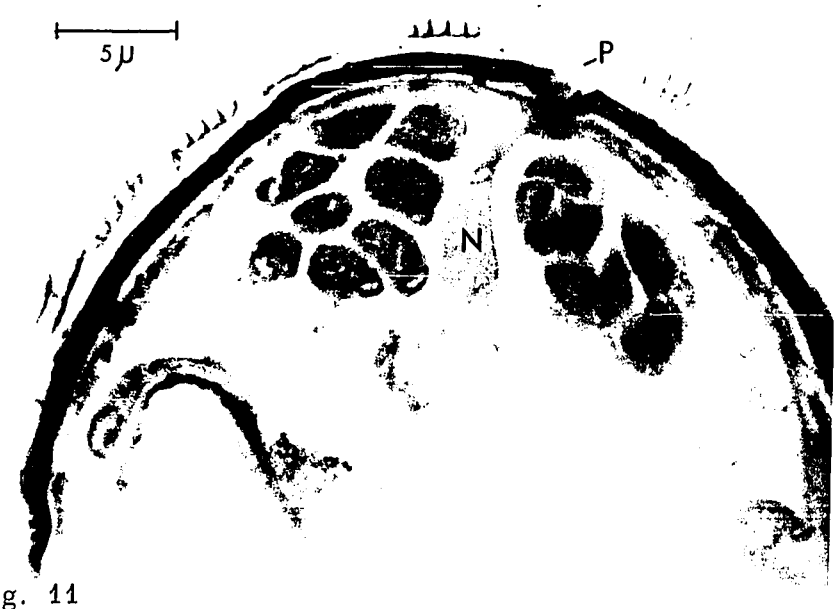

Fig. 11
Cross section through the labium of A. aegypti (L.) showing a lateral branch of the labial nerve to a sensory peg. M muscle, N labial nerve, P sensory peg.

cell in the hair receptors of the blowfly can be seen (Fig. 12A & B). The whole labellum is densely packed with cells and the labial nerve cannot be seen; the density of cells increases distally as does the density of hair sensilla. The result is that no specific neural structures can be associated with any of the hairs. The cellular material is probably not nervous tissue but is more likely displaced and cramped epidermal cells with nervous tissue mixed in and inseparable. On numerous occasions I tried vital staining with methyene blue but was unsuccessful in tracing the labial nerve to the sensilla. Methylene blue was either injected into the head or diffused through the cuticle of the labium after puncturing with a needle. The ligula and ligular hairs can be seen protruding into the lumen of the labial canal, however, no specific neural structures can be seen associated with these hairs.

1.3 Discussion:

My work on the labrum has confirmed that there are distal pegs present on the tip with nerves at the bases branching from the labral nerve. These structures resemble basiconic chemoreceptors but there is very little physiological evidence to suggest that they are (see IV). The morphological evidence suggests that these structures are chemoreceptors and in the subsequent sections of this work I intend to provide physiological evidence to clarify their function.

In studying the labellar hairs I have been unable to

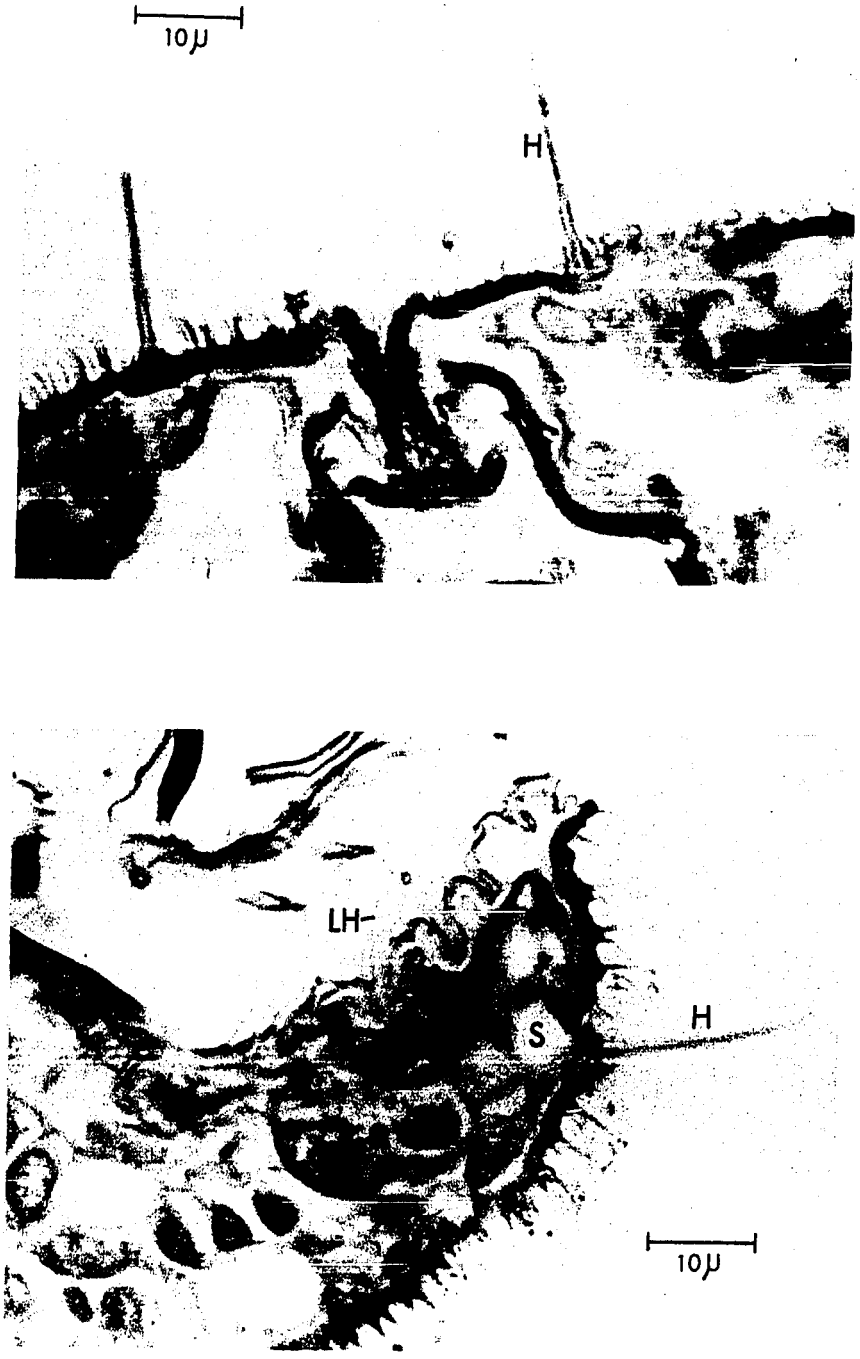

Fig. 12
Cross section through the labella of A. aegypti (L.). H long labellar hair, L ligula, LH ligular hair, S socket at the base of the hair.

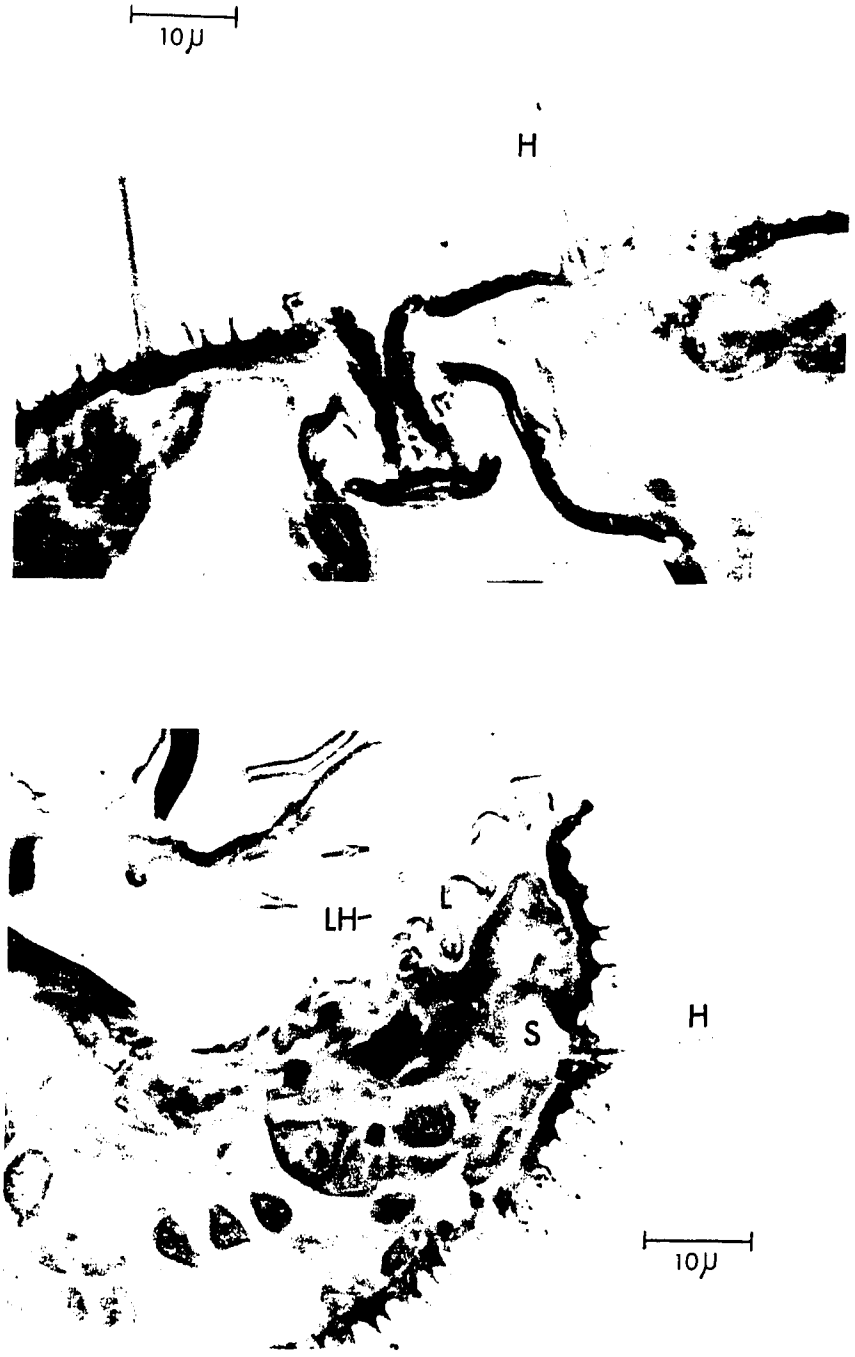

Fig. 12
Cross section through the labella of A. aegypti (L.). H long labellar hair, L ligula, LH ligular hair, S socket at the base of the hair.

determine the pattern of innervation. The hairs may be classified as sensilla trichodea and this type of sense organ can have one or more neurons associated with it and may be either thick or thin walled. My observations of whole mounts with the light microscope have led me to believe that these hairs are thick walled. With the light microscope techniques that I have used, the limitations of resolution prevent any real conclusions about the fine structure of these hairs. The presence of analogous sensory hairs on the labella of other families of Diptera, and electrophysiological evidence presented in this thesis suggest a sensory function for these hairs. Undoubtedly a study of these hairs with the transmitting electron microscope would reveal their fine structure and give a better understanding of their function.

2. Electrophysiology:

2.1. The Effect of Chemical and Mechanical Stimulation of the Labrum:

Initially, the method of combining the recording electrode and stimulating pipette was used (Hodgson and Roeder, 1956). Later, insertion of the tungsten recording electrode in the base of the labrum, and a separate stimulating pipette were used. Using both recording methods the following solutions were applied to the entire tip of the labrum: 0.5 M sucrose, pH 6.0; 10^{-2} M ATP; 0.5 M NaCl,

pH 5.6; 1.0 M NaCl, pH 5.4; 0.5 M $CaCl_2$, pH 5.8; 0.5 M $LiCl_2$, pH 5.2; 0.5 M KCl, pH 5.7; 0.5 M $MgCl_2$, pH 5.8; and 0.5 M choline chloride, pH 5.1. The tests were performed on 30 separate preparations. The solutions were always applied in the absence of mechanical deflection. There was no indication of any spontaneous activity in the labral nerve during tests nor was there any detectable electrophysiological response resulting from the stimuli. The only detectable electrical activity was an amplifier blocking artifact occurring within about 100 msec after application of the solutions. This type of artifact has been reported by Hodgson and Roeder (1962) and Gillary (1966) and has no relation to the stimulus.

After application of the above chemicals each labral preparation was stimulated mechanically by placing an empty pipette over the tip of the labrum and bending the distal 1/3. There was no detectable response to mechanical stimulation of the labrum. In addition the distal pegs were pressed head-on with a glass rod and no response was seen.

2.2 The Effect of Mechanical Stimulation of the Labellar Hairs:

The long labellar hairs were stimulated with steady and time-varying mechanical deflections. My initial method of mechanical stimulation was to deflect the hair with a capillary using a micromanipulator to control the movement.

The results of this method are found mainly in the section dealing with chemical stimuli and agree with the results in section III-2.21 (Fig. 21 & 23) but are also found in the spike frequency adaptation and spike amplitude decay section (2.211 & 2.212). A more refined method of constant length deflection using a vibration generator (described in II-3.21) driven by a feedback controlled square pulse, was used in section III-2.21.

2.21 The Response to Prolonged Constant Deflection:

Fig. 13 shows a continuous record of the response of a single long labellar hair to a constant step deflection. The length of the stimulus was 13 seconds and the deflection was 0.02 mm. An initial high frequency response during the first second can be seen, which decreases to a steady low level continuing until the stimulus is removed. I have observed the steady firing persist with a slight decay for periods up to 100 seconds. In such cases the frequency was always between 50 and 70 impulses/second initially and decayed rapidly to 20-30 impulses/second.

All of the long hairs respond in the same way to a maintained step. However, on a single preparation not all hairs respond equally well. Those hairs responding as in Fig. 13 were the only ones included in this study and for convenience only the hairs near the tip were used because of their accessibility.

2.211 Spike Frequency Adaptation:

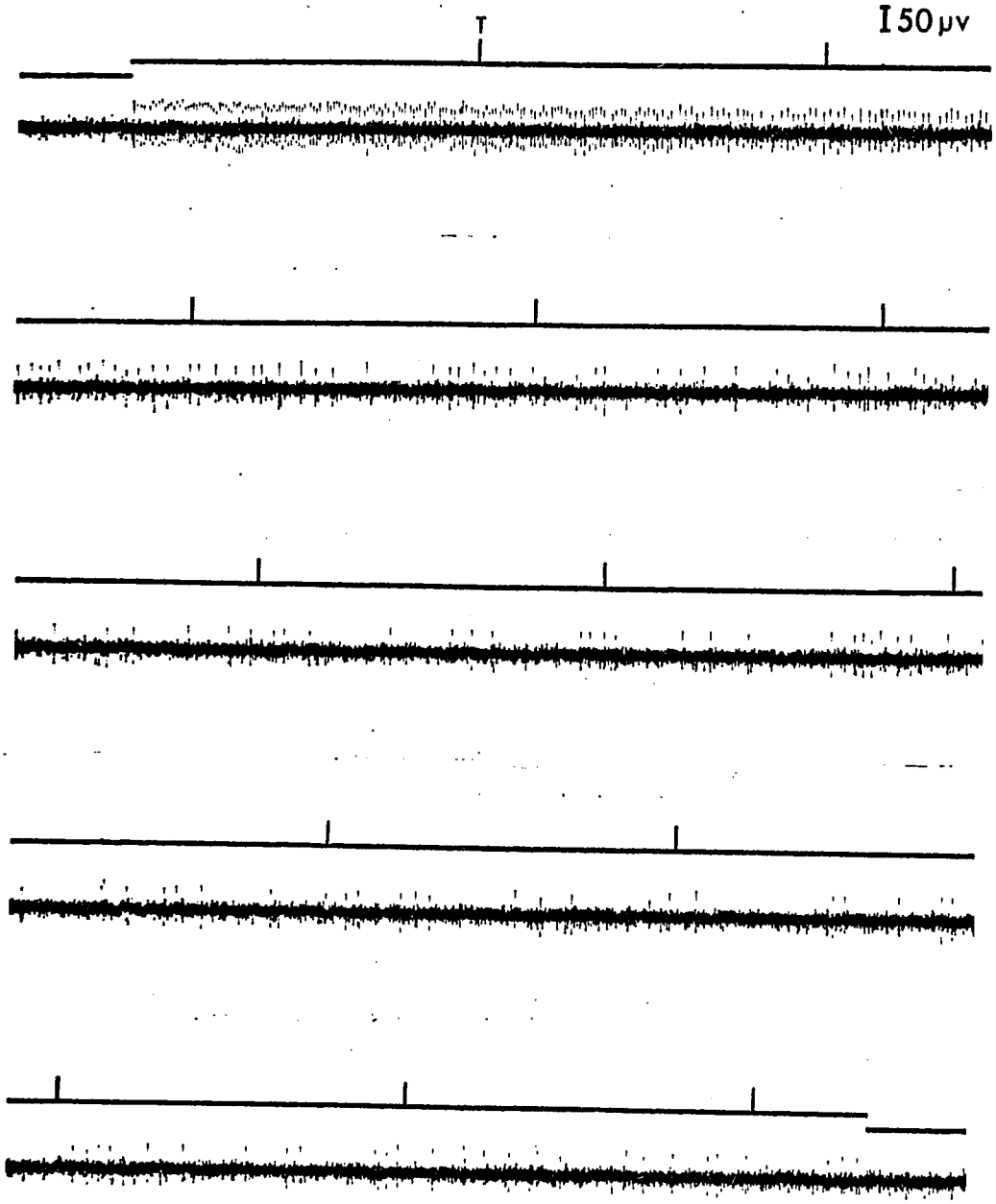

Figure 13
Response of a single long labellar hair to continuous mechanical deflection. Upper trace - square pulse to the coil and onset of stimulation. Time mark T 1/sec. Positive at the recording electrode is up.

Fig. 14B shows the frequency time response curve of three separate long labellar hairs in response to a maintained step. The stimulus in these experiments was applied with a hand controlled micromanipulator which probably accounts for the variability in the curves. Nevertheless the fairly rapid initial decay and subsequent slow decay can be seen in all of the hairs.

Fig. 15A shows the response of a single hair stimulated with the vibration generator. The input to the generator was a step and the output of the receptor approximates a single exponential decay during the first 7 seconds ($be^{-t/T}$) where e = 2.718, b is a constant and T is a constant. The hair was stimulated once every 50 seconds for a duration of 20 seconds and at a displacement of 0.02 mm. In Fig. 15B the log response amplitude, which is the log frequency, is plotted for the first 7 seconds showing the computed best fit to a straight line, i.e. , a single exponential. The linear correlation coefficient is 0.86 which is significant ($P \ll 0.01$) and indicates a good fit of the line to the data points. This experiment was done on three mosquitoes a total of 50 times on each mosquito; all three experiments were in agreement.

In an attempt to determine the recovery capability from spike frequency adaptation a single long labellar hair was deflected continuously until the steady slow decay was effected and then allowed to rest before stimulation (Fig. 16A-D). This procedure was repeated four times with

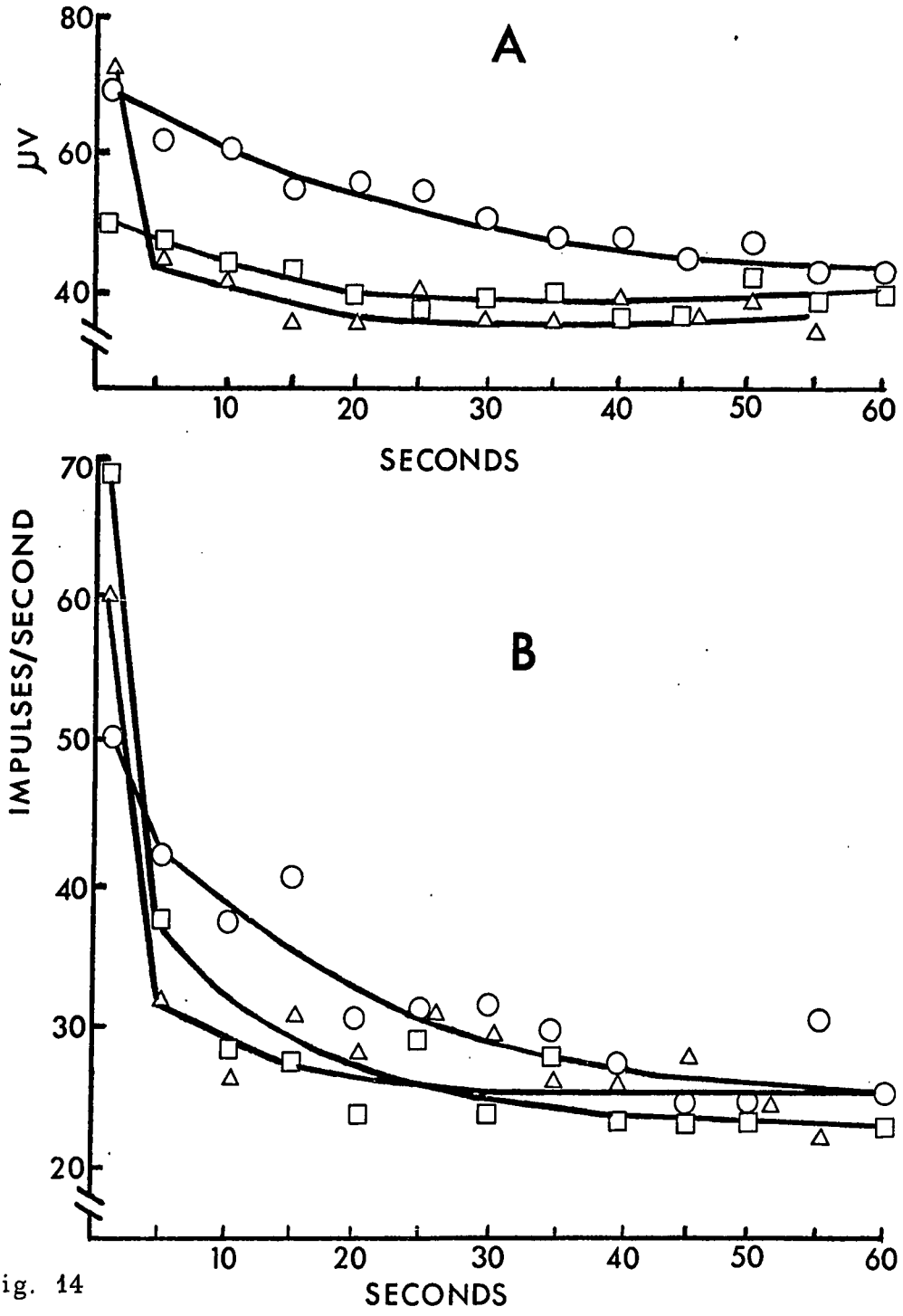

Fig. 14

(A) Spike amplitude decay curves of three separate left labellar hairs (40μ) showing the decline of spike amplitude with time during constant deflection.

(B) Time/frequency response of the same hairs as in A during constant deflection.

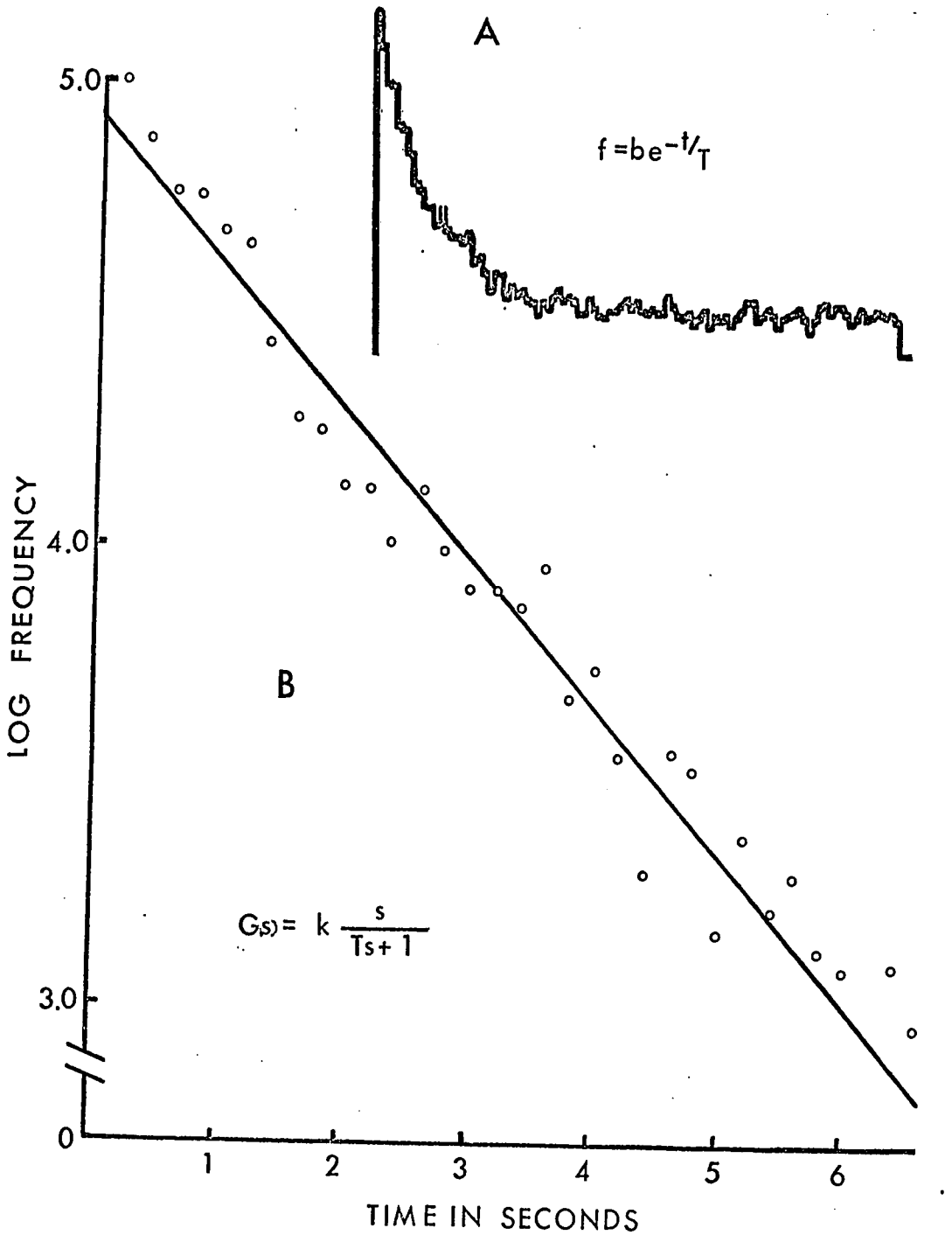

Figure 15
A'- Frequency histogram of the response of a long labellar hair to a step deflection. B - log frequency plot of the data in A.

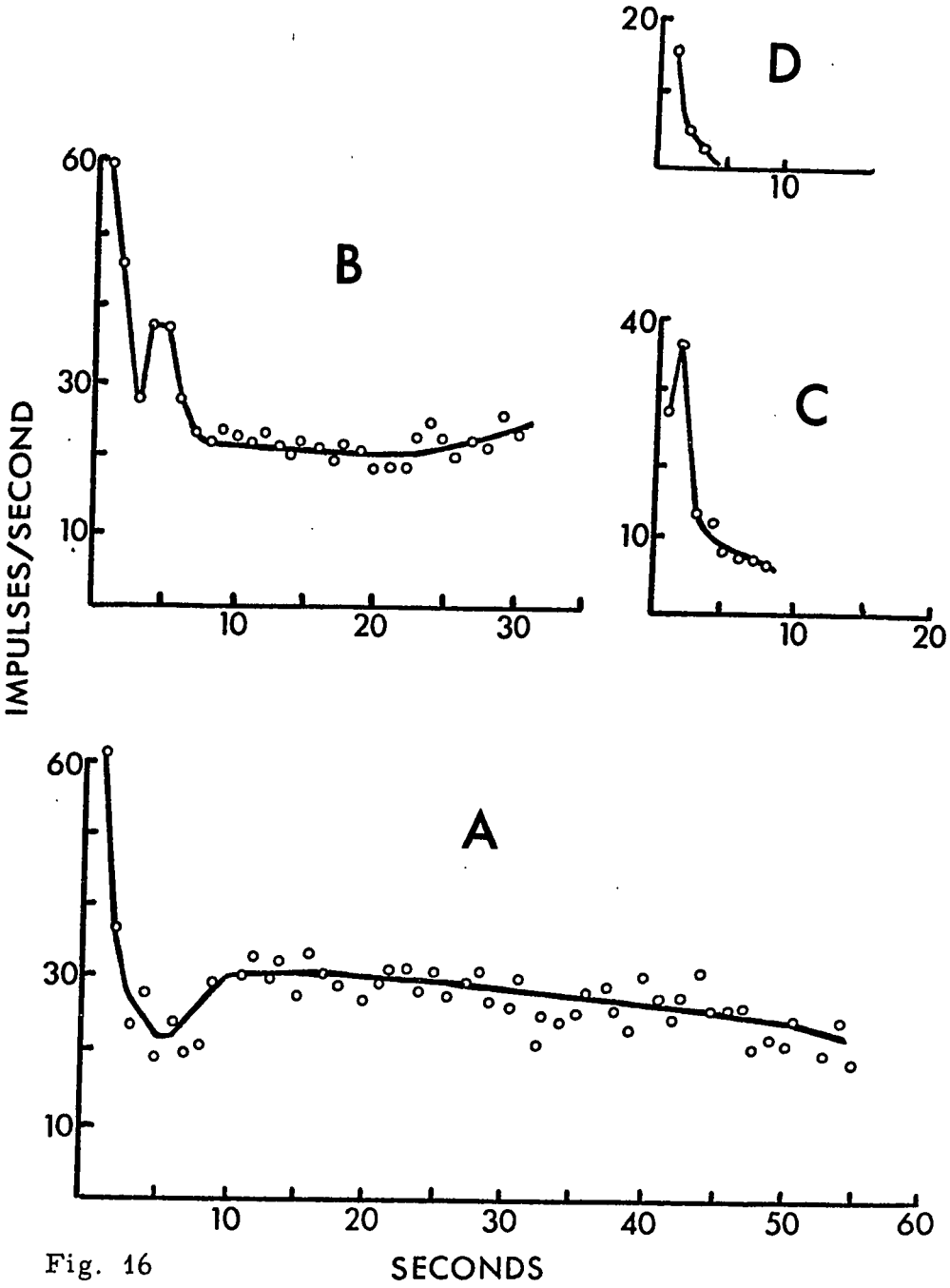

Fig. 16

Time / frequency response of a single left labellar hair to constant deflection. A - initial deflection; B- deflection 15 sec. after the end of A; C - deflection 10 sec. after the end of B; D - deflection 5 sec. after the end of C.

decreasing rest periods of 15, 10, and 5 seconds. The characteristic response was seen in Fig. 16A & B with an initial frequency of 60 impulses/sec decaying rapidly to the steady slow decay of between 25-30. In Fig. 16C the response failed to attain the original initial value and fell off rapidly to around 5 impulses/sec. In Fig. 16D after a 5-second rest, the initial value rose to less than half of that in Fig. 16C and decayed rapidly to 0. It is interesting that the firing fell off to zero after repetitive bending totaling 100 seconds yet a constant deflection for 100 seconds did not result in a decrease to zero.

2.212 Spike Amplitude Decay with Time:

Wolbarsht and Dethier (1958) and Wolbarsht (1960) report that a common characteristic of insect mechanoreceptors is variation in height of impulses. Fig. 14A shows the impulse height plotted against time for three separate labellar hairs. Each point is the average of 15 impulses. In two of the hairs the initial height is around 70μv decaying to a steady level of 50μv after 60 seconds. In the third hair there is very little decay with time. The slope of the curves fits very closely the slope for spike interval adaptation (Fig. 14B) which might be expected since it has been shown that both of these phenomena vary directly with receptor potential height (Wolbarsht and Dethier, 1958; Wolbarsht, 1960).

Fig. 17A-D is the result of an experiment similar to

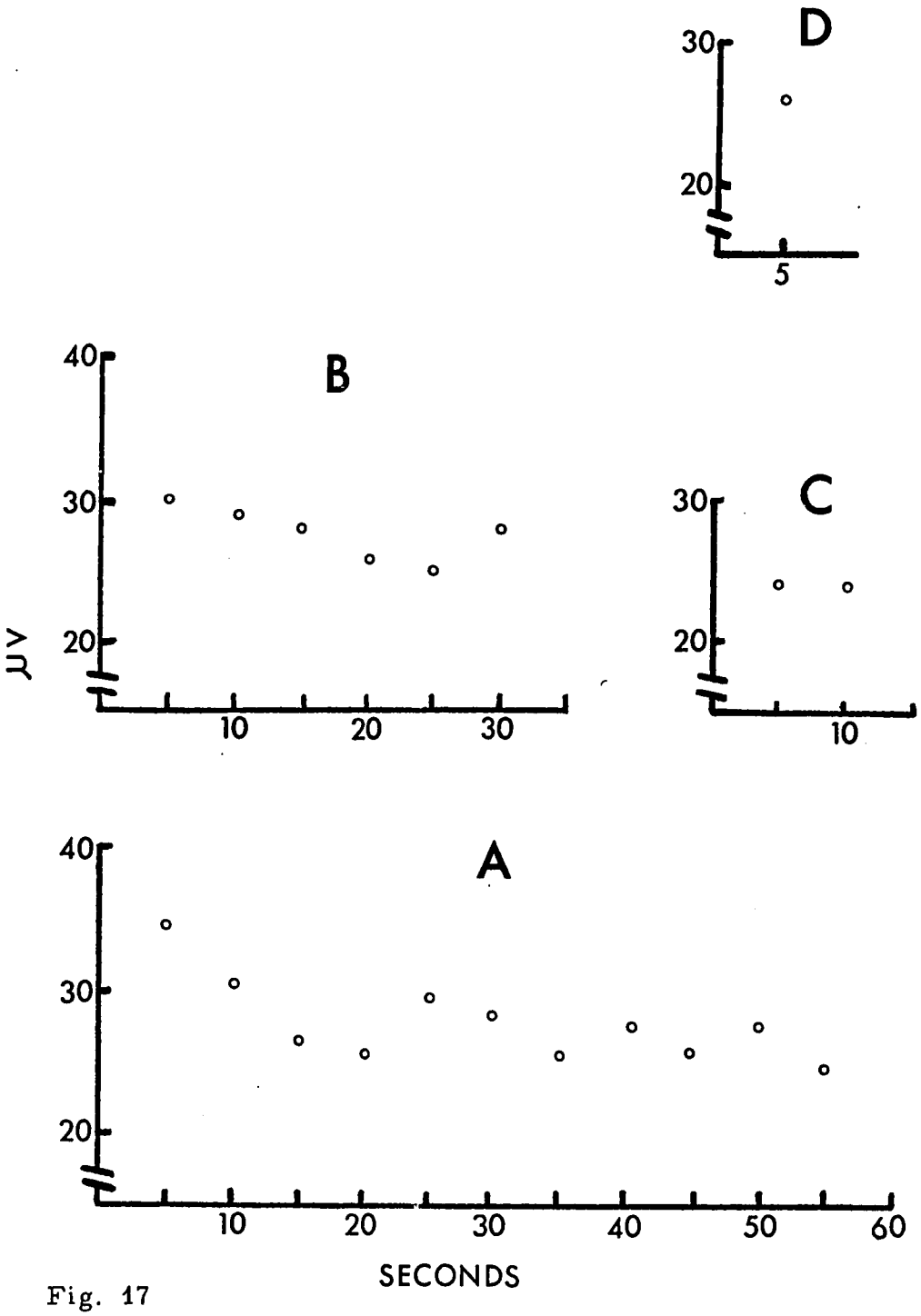

Fig. 17

Spike amplitude decay curves of a single left labellar hair. A - initial deflection; B - deflection 15 sec. after the end of A; C - deflection 10 sec. after the end of B; D - deflection 5 sec. after the end of C.

that of Fig. 16 except that the impulse height was plotted against time. Each point is the average of 15 impulses. In Fig. 17A the impulse height is slightly greater than in Fig. 17B. Fig. 17C & D are initially lower than either of the two previous trials and these results also correlate with the spike interval decay (Fig. 16).

2.22 The Response to Time-Varying Stimuli:

In section 2.21 I have established that the labellar hairs are slow adapting when stimulated by a static deflection. Since these hairs come in contact with an environmental substrate it is unlikely that they are subjected to purely static stimuli; more likely they respond to vibrations of varying frequencies. In this section the hairs are stimulated with time-varying sinusoidal deflections at various frequencies, with and without the addition of random white noise to the mechanical stimuli, in order to determine the response characteristics to phasic stimuli. No effort was made to measure the exact length of deflection because of the extremely small size of the hair. At low frequencies it was possible to keep the hair in contact through most of the cycle of the sinusoid, but at frequencies higher than 10 Hz this was impossible. The maximum movement of the stimulator at frequencies less than 5 Hz was 0.04 mm which would deflect a 40μ hair through about 42° of arc from the vertical. Since the glass capillary produced a response when just in

contact with the hair due to substrate vibration, it was kept clear of the hair through the very first part of the first cycle and then adjusted to deflect the hair through the whole cycle during subsequent cycles; analysis was started after the first few cycles.

2.221 Phase Locking as a Result of Sinusoidal Stimulation:

Nerve cells which discharge regularly tend to become phase locked to a sinusoidal stimulus (Stein, 1970). Phase locking is the confinement of the response to a particular point on a cyclic stimuli; this locking is constant for all cycles at a given frequency.

Fig. 18A-F shows the results of time-varying stimuli applied to a long labellar hair. At a frequency of 1 Hz the cell fires at about 25 impulses/cycle (Fig. 18A). At 5 Hz the cell fires at 2, 3 or 4 impulses/cycle, and at 7 Hz the cell fires at 2 impulses/cycle (Fig. 18B & C). At the rest of the frequencies the cell locks 1 : 1 to the cycle. This pattern varies somewhat among preparations but the general pattern is always the same.

In Fig. 19A-I the data is presented as a post-stimulus time histogram. Each sweep of the histogram is triggered by the square pulse at a constant point on the sine wave. The x axis of the histogram is divided into bins which vary in size dependent on the frequency. Each bin contains the number of impulses occurring at a particular point in time of the sine, and the impulse density can then be displayed

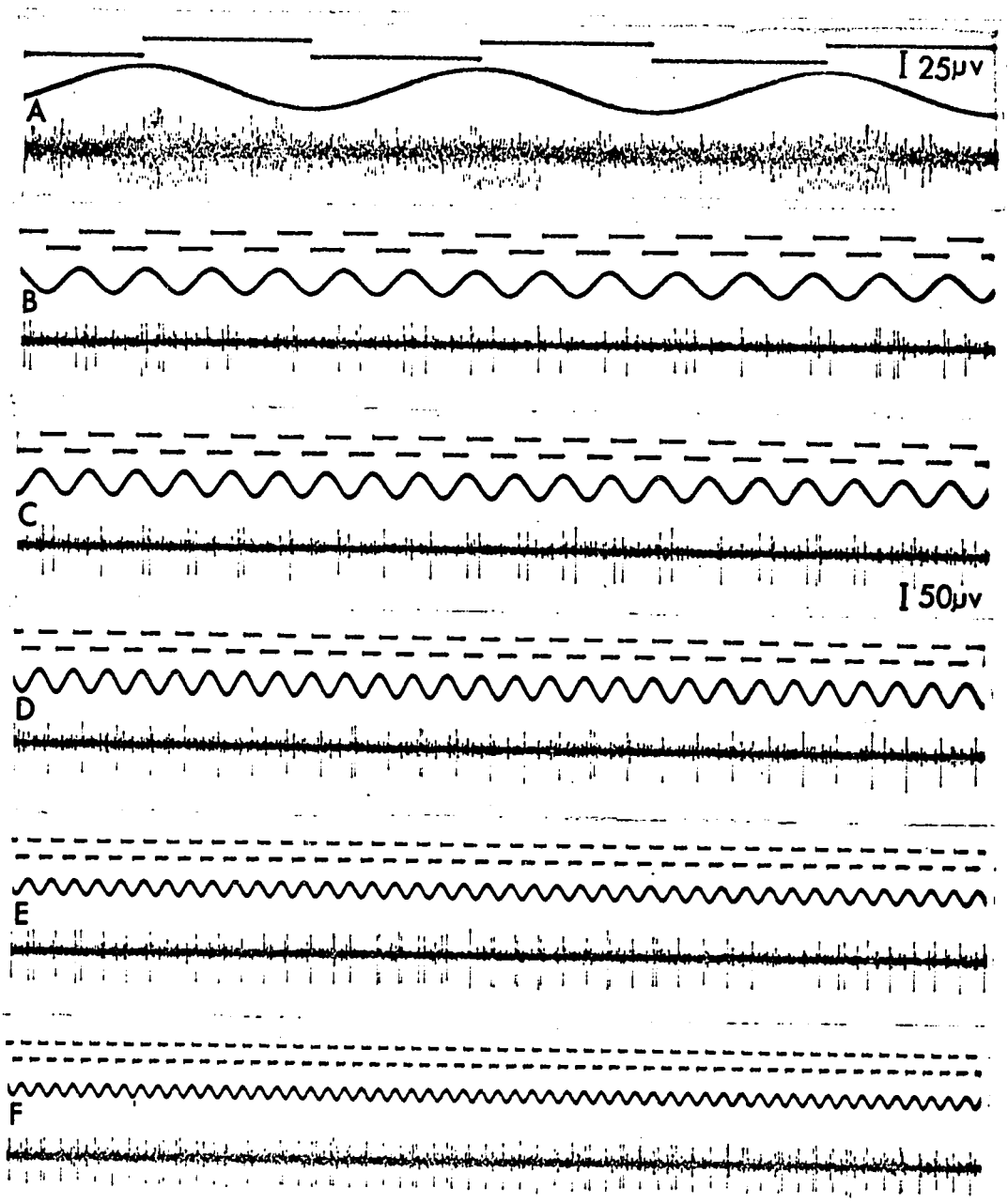

Fig. 18

Response of a single long labellar hair to time-varying mechanical stimuli, A 1 Hz, B 5 Hz, C 7 Hz, D 10 Hz E 15 Hz, F 20 Hz. Upper trace - square trigger pulse. Middle trace - mechanical sine output from the coil. Positive at the recording electrode is down. The direction of the deflection of the sinusoid is down.

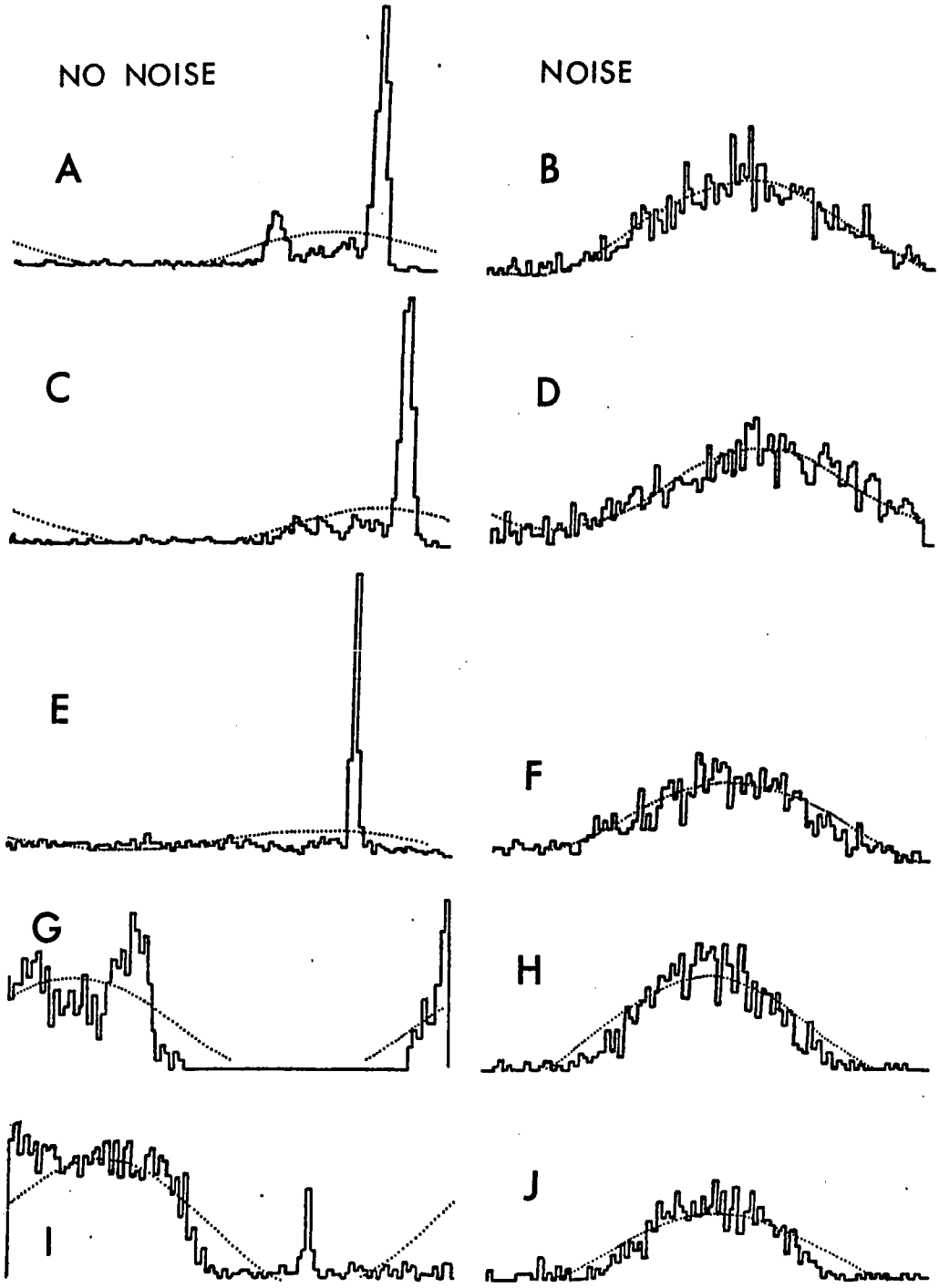

Fig. 19 Post stimulus time histograms of the response of a single long labellar hair of A. aegypti to mechanical stimuli. A & B - 20 Hz., C & D - 15 Hz., E & F - 10 Hz., G & H - 5 Hz., I & J - 0.1 Hz.

at given points after the beginning of the sine. Each histogram represents 100 sweeps of single separate sine cycles.

Fig. 19A, C, E, G & I show the post-stimulus time histogram for: 1 Hz, 10 Hz, 15 Hz and 20 Hz. The histograms show various degrees of rectification at low frequencies and phase locking at higher frequencies. Rectification is common at frequencies between .1 Hz and 6 Hz; such a response is not a sine but a clipped portion of the sine relating only part of the output with the input; at 7 Hz (Fig. 18C) the receptor phase locks at 2 impulses/cycle and at 10-20 Hz there is generally a 1 : 1 phase locking with an occasional second impulse occurring in the cycle (Fig. 18D, E, F; Fig. 19E, G & I).

Fig. 20 shows the frequency response of two labellar hairs on two separate preparations. The amplitude of the response (Fig. 20A), which is the impulse density, shows a maximum response at 3-5 Hz with the curve tailing off at both ends. The dashed curve stops at 1 Hz at the lower end but would probably follow the same slope as the solid curve if it were extended. The phase of the response in relation to the stimulus is plotted in Fig. 20C. The phase between 1-4 Hz is matched with the stimulus but lags at low and high frequencies.

2.222 The Effect of Adding Random White Noise to the Sinusoidal Input Stimulus:

Fig. 19B, D, F, H & J show the post-stimulus time histograms at various frequencies of stimulation with added noise. The distortion of the sine input by the receptor has been partially eliminated in Fig. 19H & J and completely eliminated in Fig. 19B, D & F where the nerve is responding over the entire cycle of the sine. The frequency response curves (Fig. 20B & D) for amplitude and phase relation show a slight increase in the flat portion of the response near the low (.1 Hz) and high (20 Hz) ends. The experiment represented by the dashed curve shows a depressed amplitude and more variable phase shift due to too high an amplitude of added noise. The amount of noise needed to overcome phase locking is dependent on the amplitude and frequency of the sine input. Since the noise is superimposed on the sine wave too much noise will completely distort the sine and result in a randomly changing signal. For this reason the phase relationship of the dashed experiment in Fig. 20B & D follows no particular pattern, yet because of the large amount of variability it may decay at frequencies around 7 Hz and higher. These experiments were performed on a total of four separate hairs, however, with the addition of noise only two experiments yielded results. The other two experiments without noise produced results which agreed well with the two presented graphically.

2.223 The Describing Function of the Mechanical Responses:

When information is transmitted through a physical

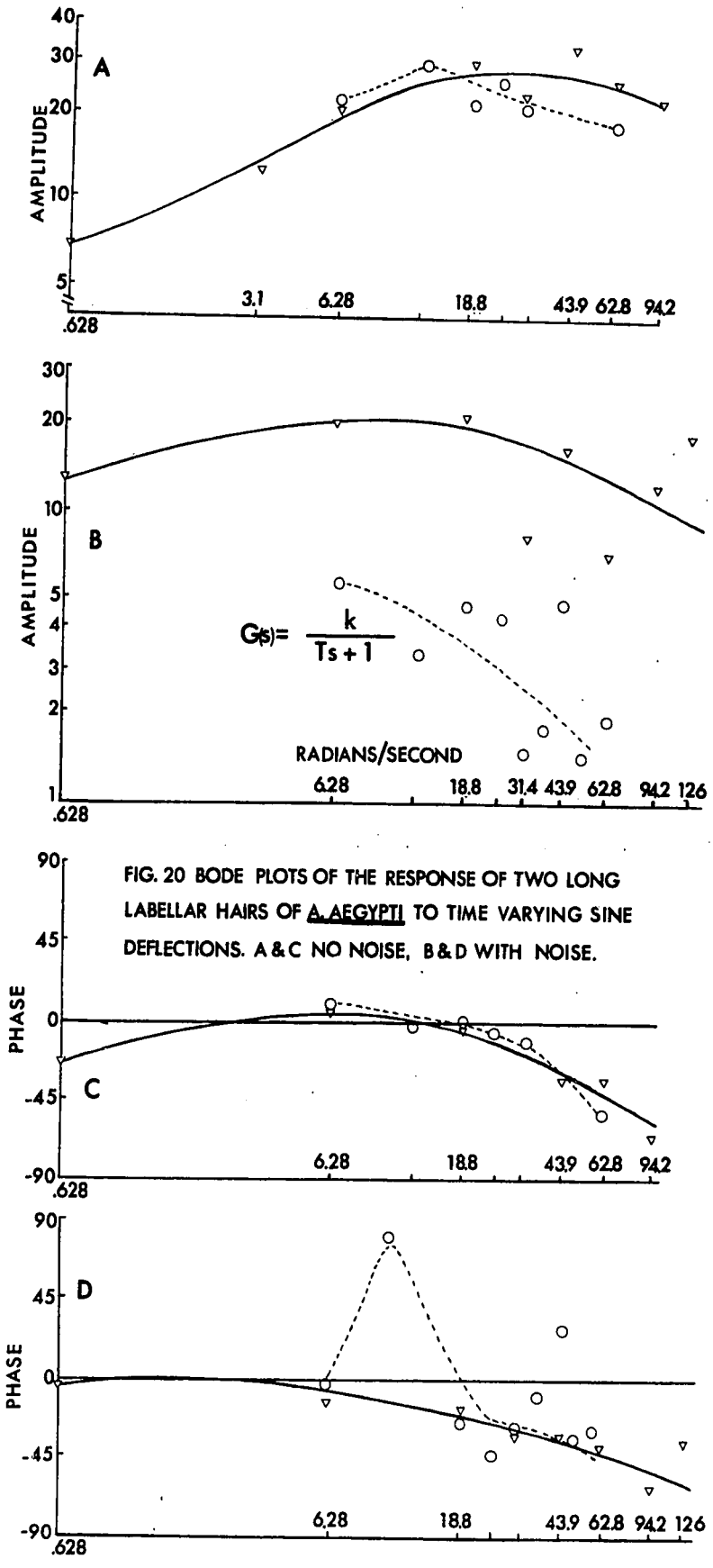

FIG. 20 BODE PLOTS OF THE RESPONSE OF TWO LONG LABELLAR HAIRS OF A. AEGYPTI TO TIME VARYING SINE DEFLECTIONS. A & C NO NOISE, B & D WITH NOISE.

system the system will have a modifying effect on the information it transmits (Garner, 1968). One way of representing this influence mathematically with a linear system is with the transfer function:

$$G(s) = \frac{\text{output (s)}}{\text{input (s)}} \qquad 1.$$

where G(s) is the transfer function and (s) is a complex frequency variable. The response of the labellar hair -- the average instantaneous frequency of impulses or the post-stimulus time histogram -- to a sinusoid is not a sinusoid but a distorted, rectified sinusoid at low frequencies and at frequencies above 7 Hz is phase locked. Thus the system is nonlinear. The transfer function can only apply to a linear system. However, the describing function technique gives the output which is linearly related with the input. Nonlinear systems such as receptor systems can be analysed providing the response has a linear part.

It is not my purpose to derive the describing functions since this has already been done extensively in several texts dealing with automatic control (DeRoy, 1966; Garner, 1968), and in works dealing with biological control systems (Milsum, 1966).

The exponential response to a step input ($be^{-t/T}$, sec. 2.211) is best described by the function:

$$G(s) = \frac{k_1 s}{T_1 s + 1} \qquad 2.$$

where s is the complex frequency variable, T is the time constant, and k is a constant derived from the slope of the exponential decay. This function describes a frequency response to a sine input with a low break frequency at 1/T. The break frequency is the point where the two asymptotes would meet. The time constants for 3 separate preparations are 1.98, 2.1 and 2.3 which averages to 2.2; therefore, 1/T = 0.41 radians/second which is the break frequency. The actual data provides a break frequency at about 10 radians/second. However, the slope of this curve may have changed with the addition of more data points. With the addition of noise there is an increase in linearity at low frequencies (Fig. 20B). This linearity suggests that the theoretical break frequency is being approached with the addition of noise.

The function for the sine input-output is,

$$G(s) = \frac{k_2}{T_2 s + 1} \qquad 3.$$

where k is the flat portion of the amplitude in Fig. 20A. This function describes the high frequency end of the curve which has an actual break frequency between 30 and 60 radians/second. If the high frequency break frequency is taken at 50 radians/second then the time constant would be

0.02 seconds.

These functions describe each end of the observed curve and may be multiplied to describe the entire frequency response:

$$G(s) = \frac{k_1 s}{T_1 s + 1} \quad x \quad \frac{k_2}{T_2 s + 1} \qquad 4.$$

There is sufficient data at the higher frequencies to show a break frequency at 50 radians/second but the low frequency response was obtained for only one preparation. The low frequency response is best determined using step inputs (Fig. 15A & B), which was replicated on three separate preparations with the time constants given above.

2.3 The Effect of Chemical Stimulation of the Labellar Hairs:

Initially my purpose was to study the response characteristics of the labellar hairs to chemical stimuli. The discovery of the response of these hairs to very small mechanical deflection presents a problem when applying chemicals since in most instances any small deflection will result in a high frequency response of the mechanoreceptor. To overcome this problem the hairs were stimulated with the chemicals both with and without mechanical deflection. In addition, variation within the mechanical response during simultaneous chemical stimulation was studied.

The method of Hodgson and Roeder (1956) (see methods

sec. II-2.12) was initially used on 25 preparations. With this method the following stimuli were used: sucrose, glucose, NaCl, and mechanical deflection. No results were obtained with this method. The results in this section were obtained by use of the independent recording electrode method described in the methods section.

2.31 The Effect of Sugars:

Solutions of 0.01 M sucrose (pH 6.35) and 0.5 M sucrose (pH 6.0) were applied to the long labellar hairs both with and without mechanical deflection (Fig. 21 & 22). In both figures the response to a mechanical stimulus is seen but there is no detectable change in the response when sucrose is applied. This experiment was repeated on 10 separate preparations; all the results were negative. Fig. 24 and 25 show the average response to 0.01 M and 0.5 M sucrose. The mean values and standard errors for these points can be found in table 2. These responses (Fig. 24 & 25) are the result of a combination of mechanical and chemical stimulation and there is no noticeable change in the response when sucrose is applied at either concentration. Solutions of 0.1 M (pH 6.2) and 0.5 M (pH 6.1) D-glucose were applied to three separate long labellar hairs without mechanical deflection and no response was seen.

2.32 The Effect of Salts:

A series of experiments was performed to determine

Table 2.: Mean values in spikes/second and standard deviations of the response of the long labellar hairs of Aedes aegypti (L.) to mechanical and chemical stimuli.

STIMULI	TIME IN SECONDS				
	1	2	3	4	5
Mechanical	69.3	49.6	44.3	25.5	27.3
n = 3	±15.55	± 2.55	± 2.55	± 3.81	± 3.08
Mechanical	40.3	31.5	33.5	25.5	27.3
n = 3	±11.64	±15.45	±15.3	±12.8	±16.4
.01M Sucrose	63.2	58.4	52.4	54.4	53.2
n = 5	± 30.37	± 15.27	± 20.86	± 27.2	± 13.61
.5M Sucrose	39.0	28.3	25.5		
n = 6	± 9.71	± 4.45*	± 9.54		
0.25M NaCl	16.5	5.0*	4.5	5.0	
n = 2	± 2.0	± 7.07	± 3.46	± 4.24	
0.5M NaCl	29.75	25.75	37.25	28.0	
n = 4	±24.56	±23.91	±21.56	±23.96	
Distilled Water	32.0	29.0	24.0	21.5	
n = 2	±29.7	±28.28	±26.15	±18.38	

* t is significant at $P < 0.1 > 0.05$

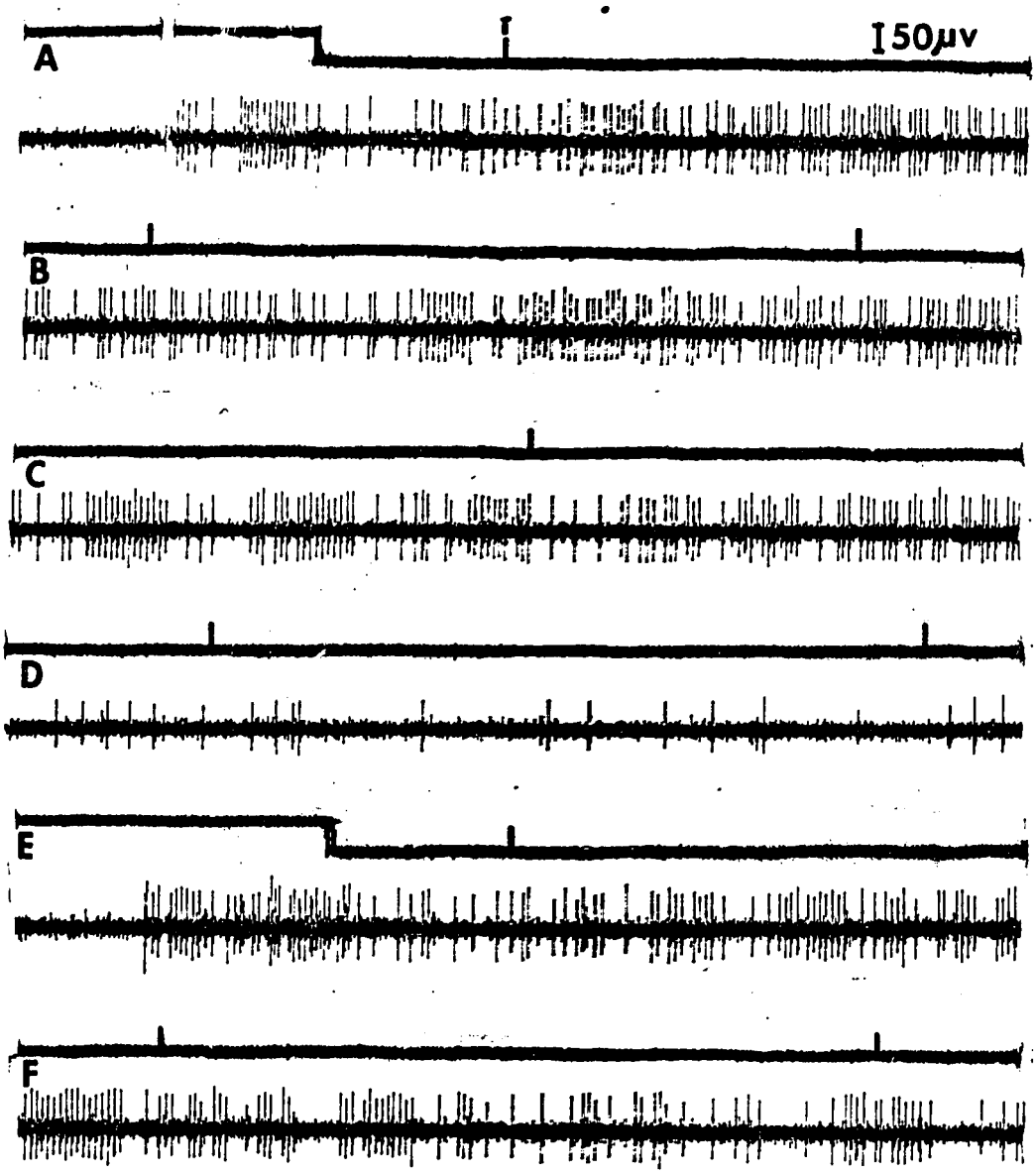

Fig. 21

A, B, C: Response of a left medial labellar hair (40μ) to mechanical deflection. first four seconds of stimulation. Continuous record.
D: Response of the above hair to deflection after 28 seconds of stimulation.
E, F: Response of the above hair to 0.5M sucrose with deflection. Continuous record.
Upper trace shows the approximate onset of stimulation. Time mark 1/sec. Positive at the recording electrode is up.

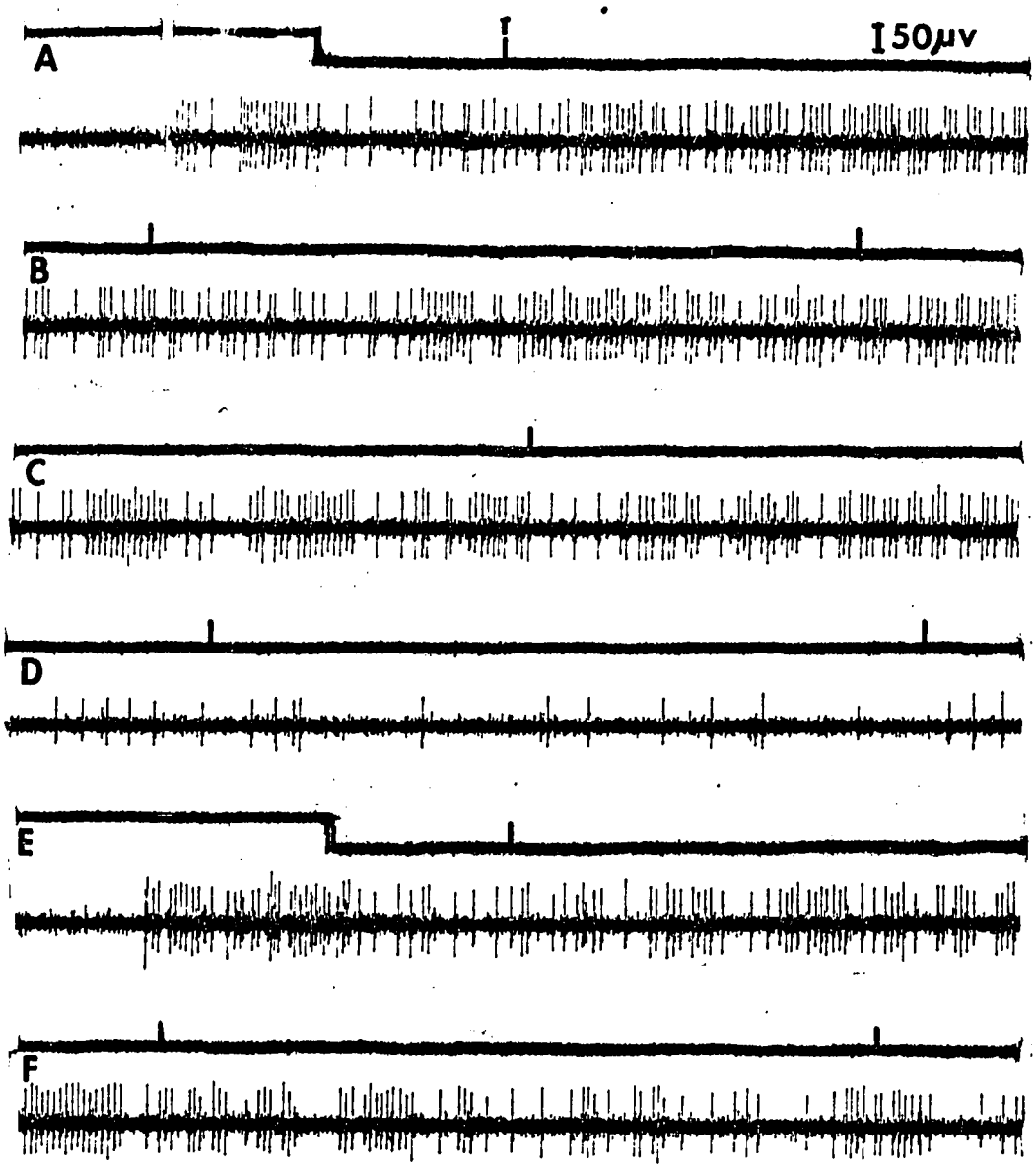

Fig. 21

A, B, C: Response of a left medial labellar hair (40μ) to mechanical deflection.. first four seconds of stimulation. Continuous record.
D: Response of the above hair to deflection after 28 seconds of stimulation.
E, F: Response of the above hair to 0.5M sucrose with deflection. Continuous record.
Upper trace shows the approximate onset of stimulation. Time mark 1/sec(t). Positive at the recording electrode is up.

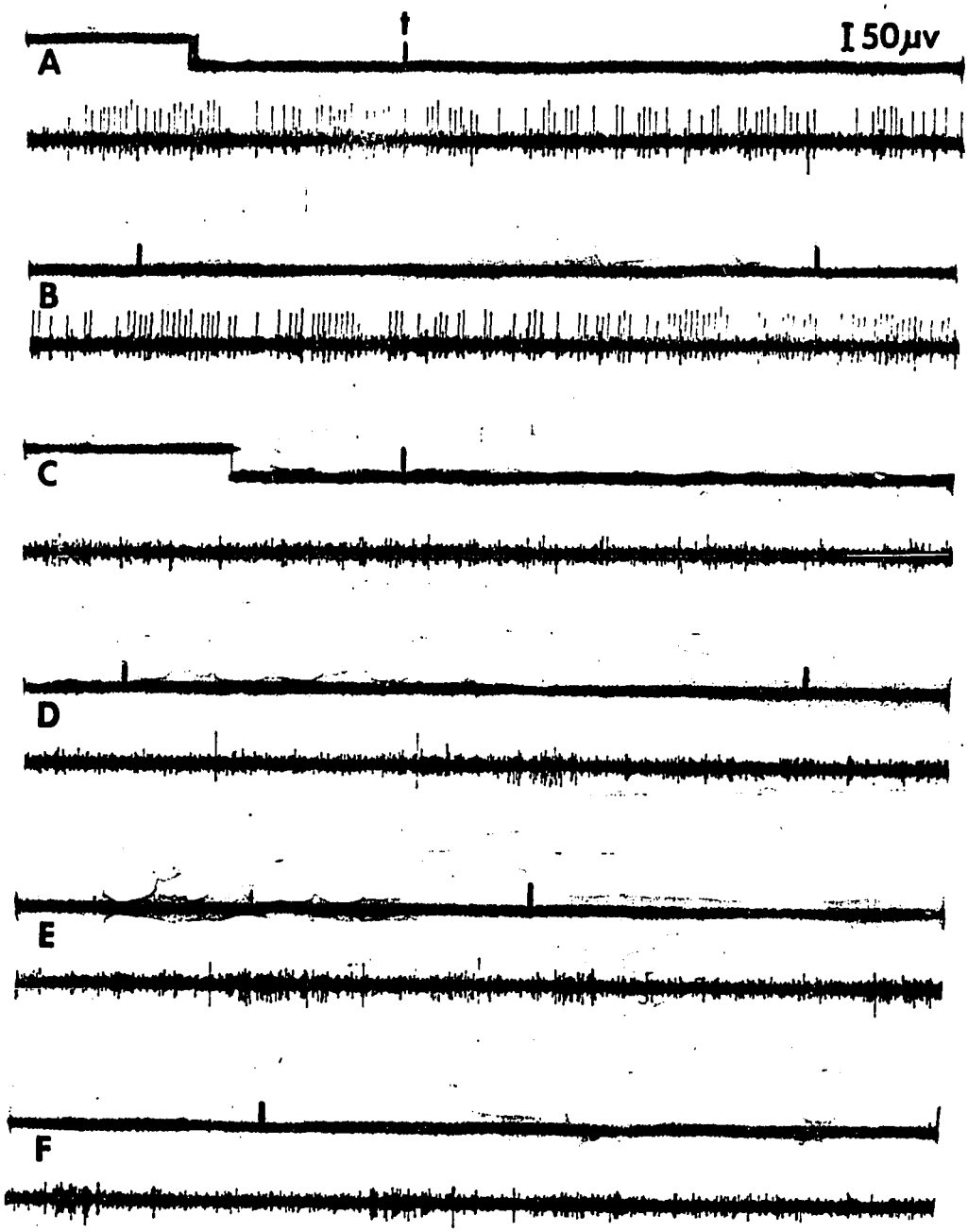

Fig. 22

A, B : Response of a left lateral labellar hair (40μ) to mechanical deflection. Continuous record.
C-F : Response of the above hair to 0.5M sucrose without deflection. Continuous record.
Upper trace shows the approximate onset of stimulation. Time mark 1/sec (↑). Positive at the recording electrode is up.

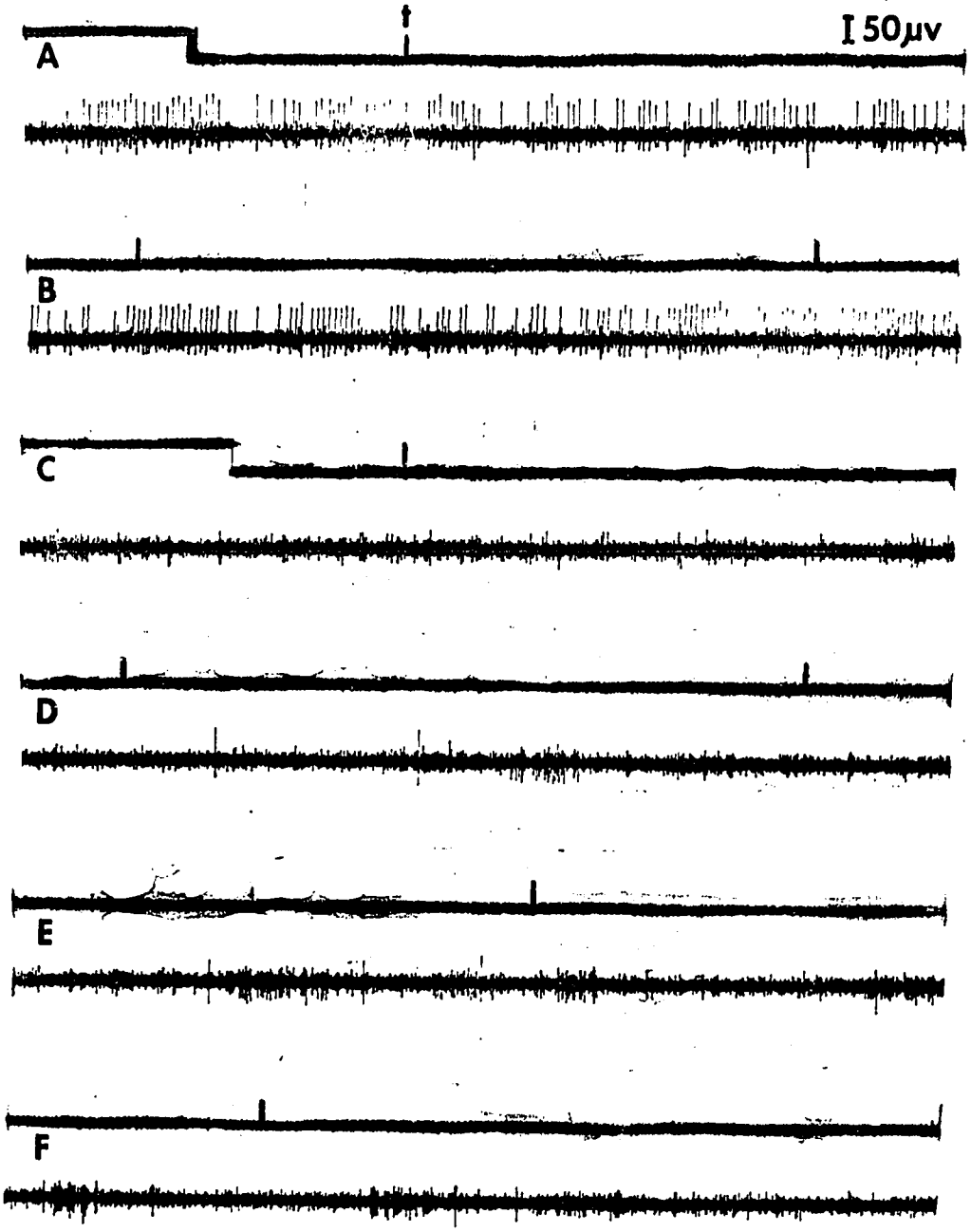

Fig. 22

A, B : Response of a left lateral labellar hair (40µ) to mechanical deflection. Continuous record.
C-F : Response of the above hair to 0.5M sucrose without deflection. Continuous record.
Upper trace shows the approximate onset of stimulation. Time mark 1/sec (↑). Positive at the recording electrode is up.

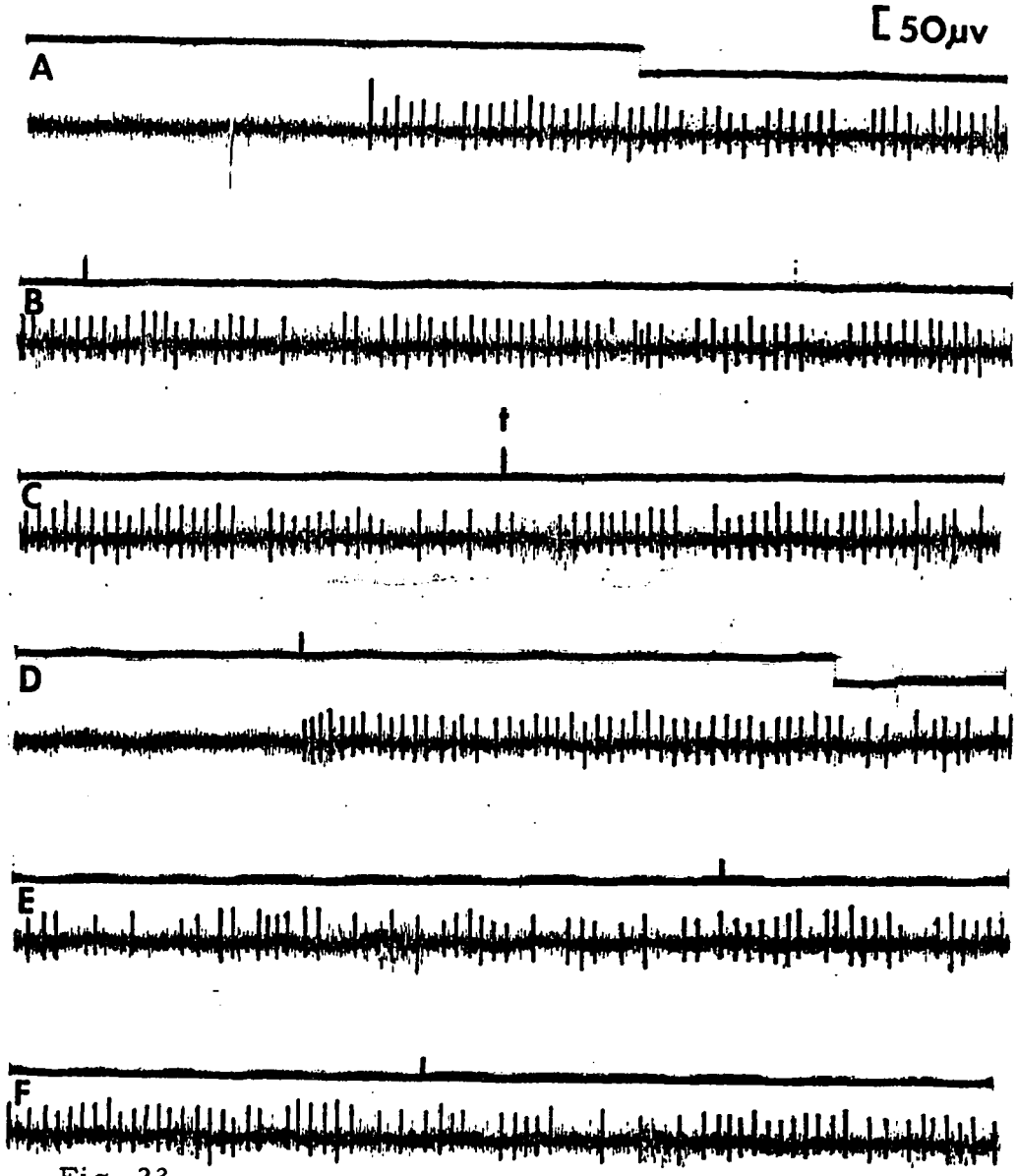

Fig. 23
A-C: Response of a left lateral labellar hair to 0.5M NaCl. Continuous record. With mechanical deflection.
D-F: Response of the above hair to mechanical deflection. Continuous record. Time mark (t) 1/sec. Positive at the recording electrode is up.

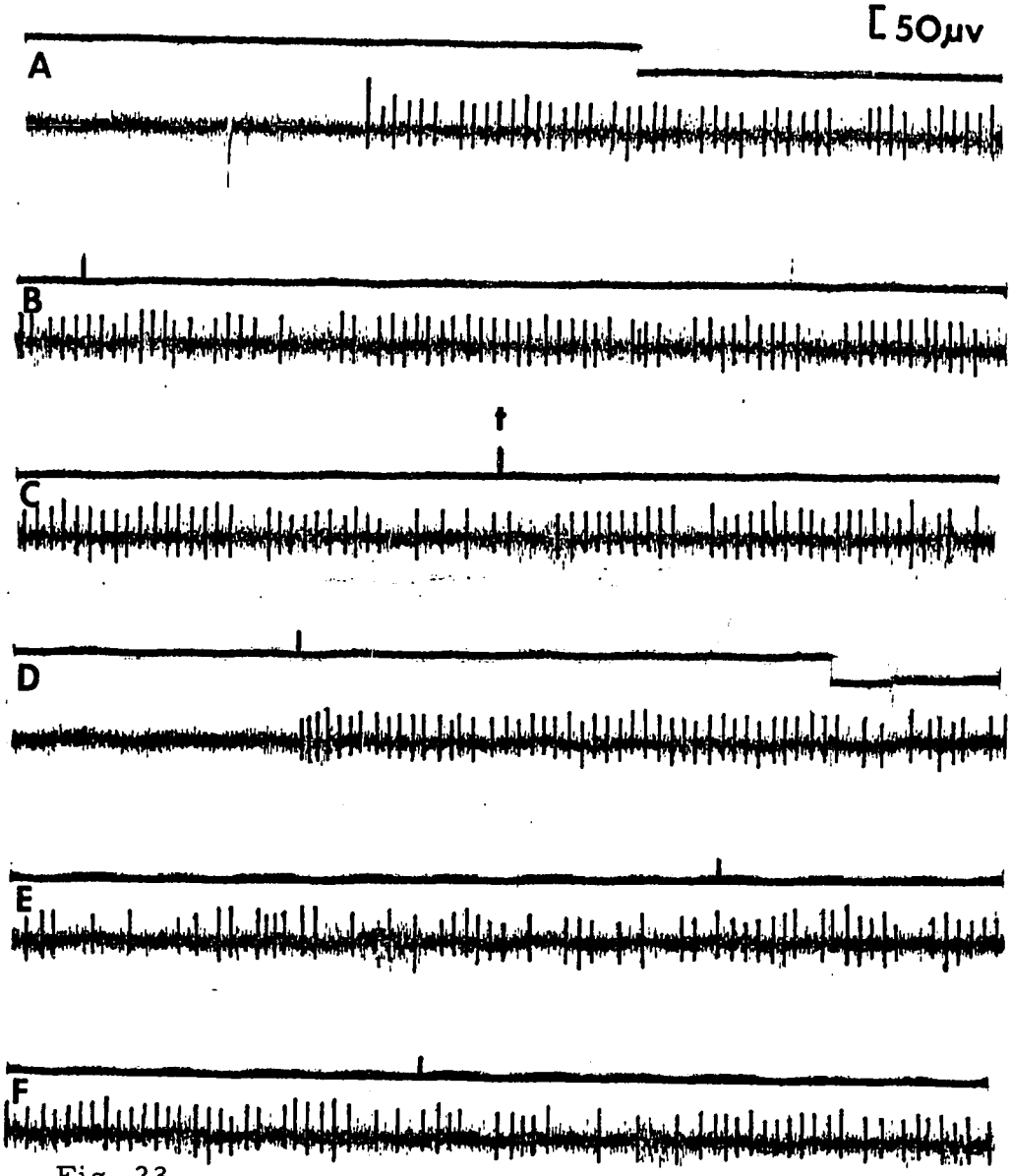

Fig. 23
A-C: Response of a left lateral labellar hair to 0.5M NaCl. Continuous record. With mechanical deflection.
D-F: Response of the above hair to mechanical deflection. Continuous record. Time mark (t) 1/sec. Positive at the recording electrode is up.

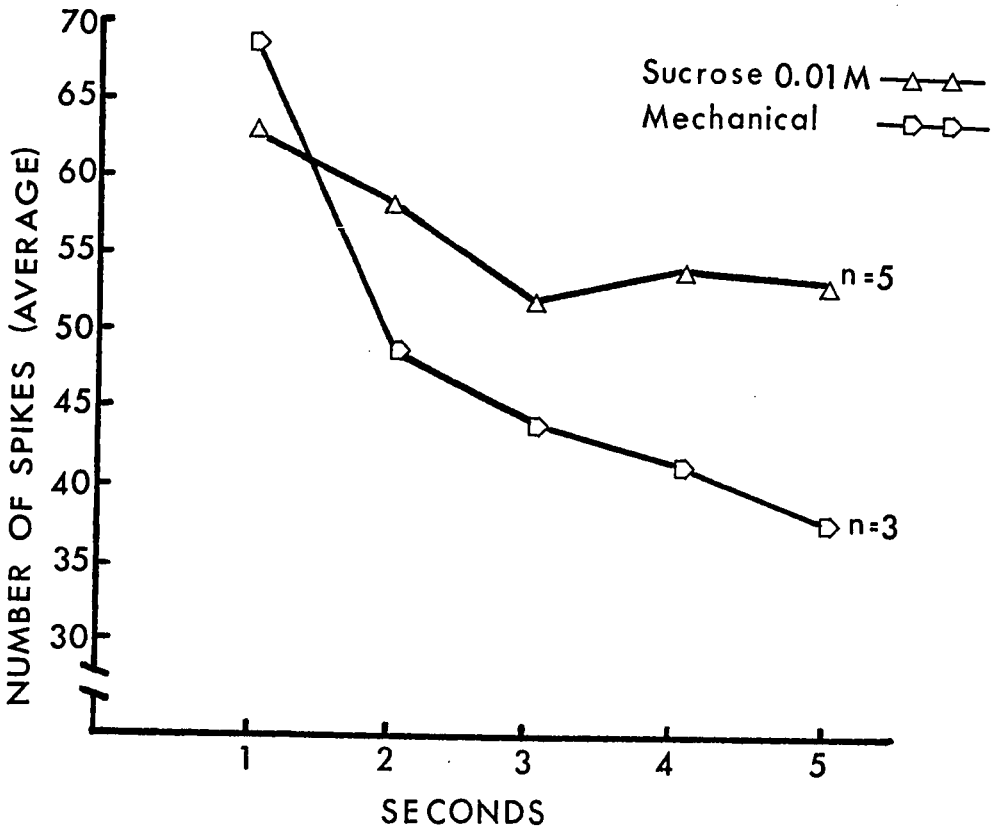

Figure 24

Response of long labellar hairs to mechanical and 0.01M sucrose stimuli. Each point is a mean of the number of trials indicated.

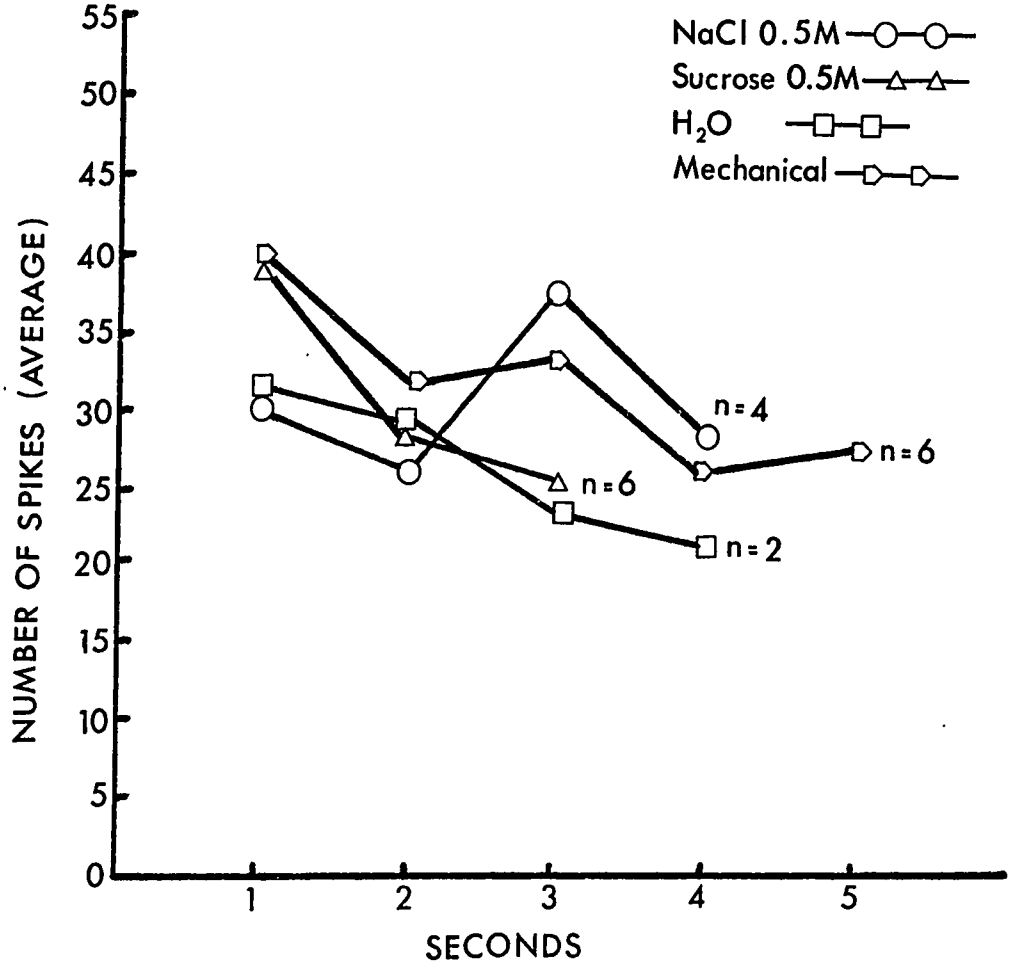

Figure 25
Response of long labellar hairs to mechanical, 0.5M NaCl, 0.5M sucrose, and distilled water stimuli. Each point is a mean of the number of trials indicated.

if the long labellar hairs respond to NaCl. Fig. 23 shows the results of mechanical deflection and 0.5 M NaCl (pH 5.6) stimulation. There is no change in the mechanical response and no additional cells discharging. Fig. 25 shows the results of four separate preparations stimulated with 0.5 M NaCl and mechanical deflection. Table 2 gives the actual mean values of impulses/second for 0.25 M (pH 5.6) and 0.5 M NaCl stimulation with mechanical deflection and in neither case is there a specific response by the receptor.

2.33 The Effect of Water:

Evans and Mellon (1962) have discovered a cell in the chemoreceptive labellar hairs of Phormia which responds solely to water. In light of this finding, I stimulated the long labellar hairs of the mosquito with water, with and without mechanical deflection, resulting in no response (Fig. 25). The mean values of the response are found in table 2. There was no significant difference between any of the means in table 2 except a slight significant difference between 0.25 M NaCl and 0.5 M sucrose for second 2 ($P<0.1>0.05$).

The application of distilled water to the chemoreceptive hairs on the labellar hairs of the blowfly Phormia regina results in complete loss of electrical activity of the L and S fibers (Hodgson and Roeder, 1956). This would mean that the water is entering the receptor area via the permeable tip of the hair and de-activating the receptor.

The water receptor described in Phormia by Evans and Mellon (1962) was unknown to Hodgson and Roeder at the time of their study and it is not known whether the mechanoreceptor of this species which was also described later, is inhibited by water. In the results presented here for A. aegypti the mechanoreceptor is not inhibited from firing.

2.34 Variations in the Early Mechanical Responses During Chemical Stimulation:

In the above sections dealing with the effect of chemical stimulation on the labellar hairs there is no indication of either a discrete specific cellular response or a response of the mechanoreceptor due to a chemical stimulus. In this section I recorded the response to chemical stimulation and mechanical deflection with the oscilloscope set to trigger on the first impulse occurring and to store one complete sweep of 10 msec. The stored sweep was then photographed, enabling me to observe all of the electrical events occurring during the first 10 msec after stimulation. This procedure was repeated on the long labellar hairs of two separate preparations using mechanical, 0.5 M sucrose, 0.5 M NaCl and distilled water as stimuli. Fig. 26A & B show the results of mechanical stimulation on the two preparations. The arrow indicates the impulses which are characteristic of the mechanoreceptor. These experiments were repeated a minimum of 10 times

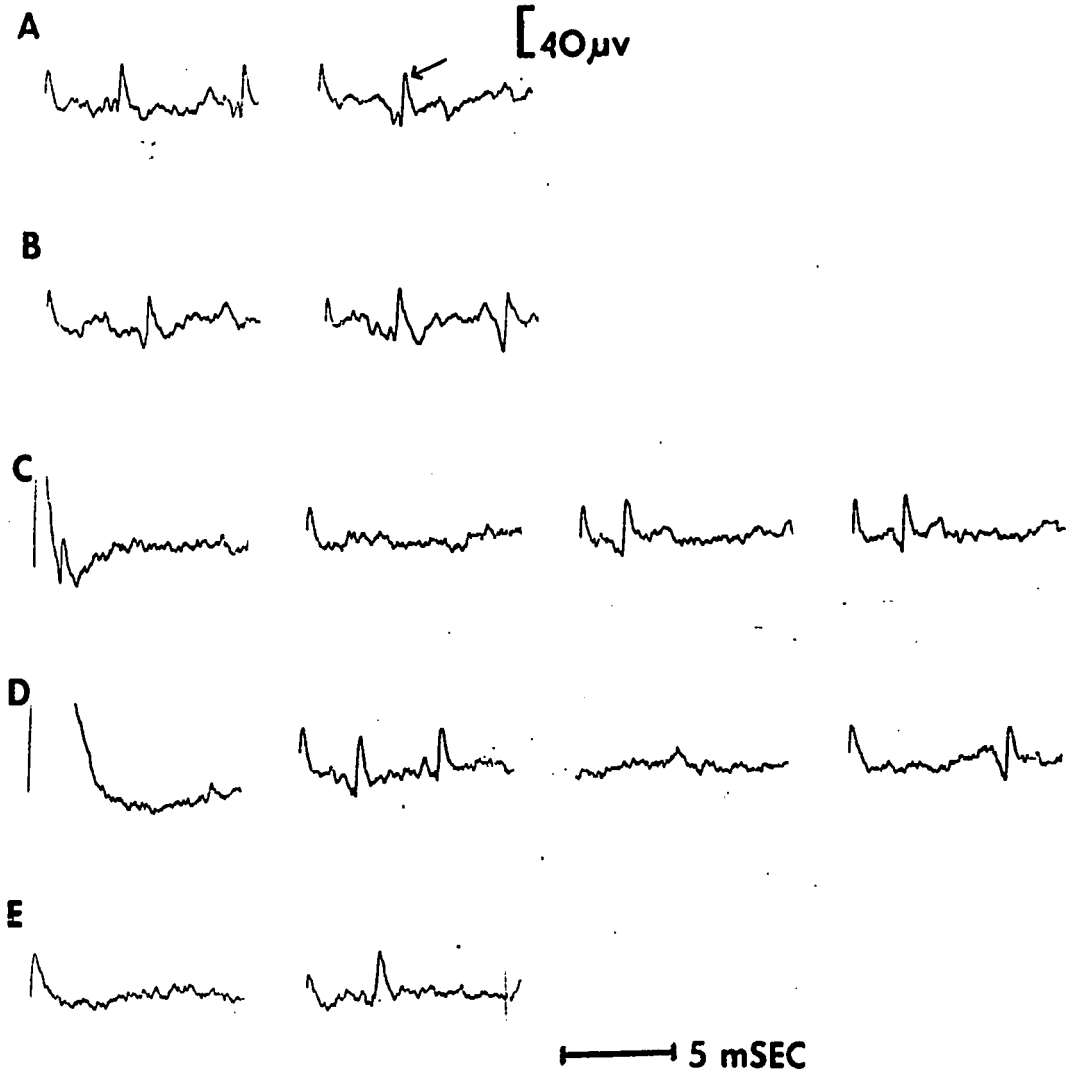

Figure 26
A: Response of a right lateral labellar hair to mechanical deflection. Prep. 122
B: Response of a right lateral labellar hair to mechanical deflection. Prep. 123
C: Response of hair(A) to distilled water with mechanical deflection.
D: Response of hair (A) to 0.5 M sucrose with mechanical deflection.
E: Response of hair (A) to 0.5 M NaCl with mechanical deflection. Positive at the recording electrode is up arrow indicates mechanoreceptor impulse. Space between frames = 5 min.

and up to 40 times in some cases. The space between frames represents five minutes.

It is interesting to note the consistent shape of the impulses from preparation to preparation and also the occurrence of the second impulse in all four trials 3 - 3.5 msec after the first. This gives an initial frequency of 300/second. Some variations in the shape of the impulse might be expected between preparations when using extracellular techniques, but the initial frequency or interspike intervals should be similar.

Fig. 26C, D & E show the result of stimulation with distilled water, sucrose, and NaCl. Other than the initial artifact in the first frame of C & D (see sec. III-2.1) no electrical activity other than the mechanical response is seen.

IV. DISCUSSION

The role of the apical labral sensilla of mosquitoes as sense organs was first suggested from morphological studies by Vogel (1921) and later by Robinson (1939) and Von Gernet and Buerger (1966). Further studies of the labrum with the scanning electron microscope by Hudson (1970) and reported here, also suggest a sensory function of the apical sensilla based on structure and innervation.

Early investigations into the mechanism of distinguishing sugars from blood resulting in the diverting of blood to the midgut and sugars to the oesophageal diverticula (Day, 1954; Hosoi, 1954; Trembley, 1952) led to speculations about the sensory functions, if any, of the labral sensilla. Day (1954) suggested that the cibarial receptors played the key role in blood identification, but Hosoi (1954) demonstrated that stimulation with whole rabbit blood and rabbit erythrocytes after amputation of the distal third of the labrum resulted in a lowering of the response. Hosoi (1959), however, assigns the major function of blood identification to the cibarial receptors and states that: "The labrum is no longer considered as bearing any specific chemoreceptors." Owen (1963) applied solutions of 1 M, 2 M, and 3 M sucrose, 2 M ammonium chloride, and water to the unsheathed stylets of Aedes dorsalis and Culiseta inornata without demonstrating any behavioral response. Owen further states that blood flow

up the food canal by capillary action resulted in active aspiration when the blood column reached the area of the cibarium, suggesting that the detection of blood takes place in the cibarium not at the tip of the labrum. Salama (1967) contrary to Owen found with Aedes aegypti that the tip of the labrum is sensitive to blood and that distal amputation of the labrum results in a decrease in sensitivity, as did Hosoi (1954). There is an apparent discrepancy in the behavioral results regarding the sensory function of the labrum. Owen (1963) describes the spontaneous aspiration of fluids by the unsheathed stylets of C. inornata occurring at irregular intervals. I have observed spontaneous aspiration occurring in Aedes aegypti accompanied by cibarial pumping. With the electrophysiological technique I used, the cibarial muscle potentials were observed firing in correspondence with fluid movement in the food canal. This activity persisted in the majority of preparations and did not change in frequency when test solutions were applied to the labrum. With frequent spontaneous activity and capillary action alone, in the absence of spontaneous activity, it is possible that any solution coming in contact with the tip of the labrum would reach the cibarium where either acceptance or rejection is mediated.

In light of the results presented in this paper I have to agree with Hosoi and Owen and assign a nonsensory function to the labral structures when stimulated

mechanically and with the chemicals used in sec. III-2.1. There are two possible explanations for the presence of these structures: 1) they are vestigial receptors once functioning as detectors of foods but no longer capable of performing this function, and 2) they are chemoreceptors but the response to chemicals has not been measured adequately. This latter possibility seems unlikely since Hosoi and Owen used experimentally sound behavioral techniques and the work presented here (sec. 2.1) uses electrophysiological techniques which should have indicated a receptor function if it existed.

The location and function of insect sense receptors has been reviewed by Dethier (1963) and for chemoreceptors of Diptera by Frings and Frings (1949). Frings and Hamrum (1950) have described contact chemoreceptors of Aedes aegypti on the labella and tarsi. They report that the tarsal receptors of A. aegypti are sensitive to sucrose. Sensitivity of the tarsal receptors, of this species, to salinity when ovipositing, has been reported (Wallis, 1954). Of interest here are the labellar hairs which are described as being of three sizes: 1) 7μ short, spread over the entire surface of the labella, 2) 20μ medium, present only on the tip of the lobes and thought to be chemosensory, and 3) 40μ long, set in rows over the entire surface of the labella and thought to be tactile (Frings and Hamrum, 1950). The shorter 7μ type are spines without basal sockets and are probably not sensory (Fig. 9). Owen (1963) working

with C. inornata describes two sizes, a 60-80μ long group and a 32-40μ group of short hairs. He states that all hairs more than 32μ in length are chemosensory. The results of this study, showing no sensitivity of the long hairs of A. aegypti to chemicals but sensitivity to mechanical deflection, agrees with the conclusion of Frings and Hamrum. This conclusion, however, differs from those of Owen and raises a question about the homologies of these hairs between genera in the family. One would expect that if the long hairs are homologous, i.e., derived from a common source embryologically, then they would have a similar function, but this is not the case.

Salama (1966) describes the taste sensitivity of A. aegypti and Rodnius prolixus to alcohols and salts using a membrane feeding technique. Salama (1967) lists the taste sensitivity of A. aegypti to sugars and salts with a technique of placing a stimulating chemical within a capillary over the tip of the labium and stylets. Salama (1967) concludes that the labellar sensilla are sensitive to sugars, water, and may be sensitive to unacceptable compounds. A correlation of the nutritional state of the mosquito with acceptance or rejection has been reported. (Galun and Fraenkel, 1957; Salama, 1969). The work of Salama (1966, 1967 & 1969) relied on the rate of cibarial pumping and degree of engorging as behavioral criteria whereas others relied on specific movements of the mouthparts as a sign of either acceptance or rejection (Frings

and Hamrum, 1950; Feir, et al., 1961; Owen, 1963). The behavioral criteria orignally established with A. aegypti by Frings and Hamrum, consisted of a labellar response, spreading and opening of the labellar lobes with cibarial pumping activity, signaling acceptance. Rejection of a compound was signaled by withdrawal of the entire proboscis. I have observed that both of these responses, especially cibarial pumping can be duplicated with mechanical deflection of the labellar hairs alone. This observation is also made by Owen (1963) yet he used these criteria exclusively in his studies with C. inornata.

From the results of my work with the long labellar hairs of A. aegypti I conclude that they are sensitive only to mechanical deflection. Since these hairs respond to very minute mechanical deflections which result in proboscis movement, the behavioral results obtained by relying on proboscis movement should be reconsidered. In my experience it is very difficult to apply a chemical to one of the long labellar hairs without evoking a response from the mechanoreceptors.

The method of food identification by mosquitoes remains uncertain. The feeding stimulants in blood for Culex pipiens and A. aegypti have been identified (Hosoi, 1959; Galun, et al., 1963), using a membrane feeding technique which excludes stimulation of labellar hairs. The labellar hairs have been found to be insensitive to blood stimuli (Hosoi, 1954), yet some authors have reported

a response to other chemical stimuli, (Frings and Hamrum, 1950; Hosoi, 1959; Owen, 1963). When the labellar hairs are stimulated with sugar and the stylets are simultaneously stimulated with blood, the blood will go to the diverticula rather than the midgut (Hosoi, 1959). This suggests that the labellar hairs are overriding the cibarial receptors which are considered responsible for directing blood to the midgut. It is obvious that the cibarial receptors play an important role in food identification and not until their function is studied will there be a more complete understanding of the method of food detection and identification by mosquitoes.

It is appropriate at this time to consider the sequence of events which lead up to the act of both blood and sugar feeding taking into account, as a summary, the role of the sense receptors involved.

The first event to occur which may be considered as specific to feeding is attraction to the source of food from a distance. In the case of blood an animal is involved as a host and the reason for feeding is quite different from that of feeding on nectar. Many causes for attraction to an animal host have been postulated the most common being vision, and chemical odorants, including CO_2, and water; heat may also be considered as an important factor since most mosquitoes prefer homiotherms. The antennae are thought to possess the chemoreceptors involved in attraction. In sugar feeding, flower scent, moisture,

heat and visual stimuli are likely attractants.

The next event to occur after the mosquito has been attracted to a food source is landing and location of a feeding area. Landing might suggest the presence of locomotion arrestant stimuli, but this need not be the case. After landing, probing is the most commonly observed behavior and undoubtedly contact chemoreceptors on the mouthparts and legs become important in locating a suitable feeding area. It is here that a major difference between the sequence of events occurs. In sugar feeding, be it a flower or artificial source, the location of a suitable feeding area coincides with the detection of the food which is followed by feeding. The detection of the food by chemoreceptors is important but where this actually occurs is unclear. As mentioned earlier it is possible that feeding is initiated through cibarial pumping and food identification occurs in the cibarium after partial ingestion.

In blood feeding, location of a feeding area is not clearly understood. A mosquito will probe on the skin of a host and then begin to pierce. Perhaps the labellar hairs are important in determining a suitable area of specific texture and shape. Piercing, the next step in blood feeding, is a complicated process beginning with cessation of probing and then insertion of the stylets to a point where either a blood vessel is entered or a pool of blood within the tissue is formed. It is here that the

labral apical structures have been associated with blood detection. It should be remembered, at this point, that the labellar hairs, as in sugar feeding, are resting on the substrate and undoubtedly responding to a variety of mechanical deflections relating to the position of the mouthparts. The next step is cibarial pumping and feeding. It is possible, in face of the evidence presented here, that detection of blood, as with sugars, takes place in the cibarium. Finally blood feeding is ended by withdrawal of the stylets. The cessation of feeding is thought to occur because of abdominal distension stimulating abdominal stretch receptors (Clements, 1963).

The evidence presented in this paper suggests a nonsensory function for the labrum and a mechanosensory function for the long labellar hairs. Since the short labellar hairs have not been studied it is possible that they mediate a chemical response. The fact that the long labellar hairs of other species respond to chemicals and those of A. aegypti do not, raise questions about the validity of comparative assumptions made for other species. It is possible, though, that other species possess very sensitive mechanoreceptors on the labella and they may, as in the case of the blowfly, be accompanied by chemoreceptors within the same hair. The extreme sensitivity of the long labellar hairs of A. aegypti is to be expected because of their function, since it is important that very minute deflections be registered during feeding. The result is

that information is transmitted about the relative position of the labella, which are spread during both types of feeding, to the substrate allowing for continual adjustment of the position of the mouthparts according to substrate movements, a system necessary for prolonged feeding on a constantly moving substrate.

Wolbarsht (1960) puts insect mechanoreceptors into two major categories based on the rate of adaptation: the first type is phasic or fast adapting signalling only an on or off response. A vertebrate example of this first group is the Pacinian corpuscle, a receptor which responds to high velocity pressure changes (Gray and Sato, 1953; Lowenstein, 1959). An insect example is the mechanoreceptor hair on the wing of Sarcophaga responding to high velocity deflections (Wolbarsht, 1960). The second type is slow adapting and signals continuously during static stimulation. Examples of this type are the crayfish stretch receptor, the mechanorecptor in the labellar hairs of Phormia, and insect abdominal stretch receptors (Lowenstein and Finlayson, 1960). The mechanoreceptor in the long labellar hairs of A. aegypti clearly belong to this second category. Even though the hairs are classified as slow adapting there is considerable adaptation evident during the first 7 seconds of stimulation.

As mentioned above these receptors are grouped according to their rates of adaptation. Adaptation is most commonly defined as a decrease in spike frequency during a

continual stimulus, but it may be defined as the decrease in magnitude of the generator potential during sustained stimulation (Patton, 1968). The generator potential is a graded, non-propagating potential, originating at the receptor terminal which gives rise to the all or nothing spikes and varies directly with the stimulus intensity. As a result of this, the spike frequency as well varies with intensity. The generator potential has been measured in many preparations: the frog muscle spindle (Katz, 1950a, b), Pacinian corpuscle (Alvarez-Buylla and Ramirez de Arellano, 1953; Gray and Sato, 1953), stretch receptors of Crustacea (Eyzaguirre and Kuffler, 1955a, b) and in insect mechanoreceptors (Wolbarsht, 1960). In the work presented here the magnitude of a stimulus could be taken as either the rate of deflection or the amount of displacement. As mentioned before the hair responds maximally regardless of direction of bending or amount of displacement, however, the impulse frequency increases with increase in the frequency of time-varying stimuli. It can be assumed from this that the magnitude of the generator potential also increases with stimulus frequency. There is often confusion involving the use of the terms generator potential and recptor potential and I use the terms as defined by Davis (1961).

Spike adaptation (Nakajima and Onodera, 1969a) and generator adaptation (Nakajima and Onodera, 1969b) have been dealt with in detail on the crayfish fast and slow

stretch receptors. In the slow adapting receptor they conclude that overall receptor adaptation is due to generator adaptation rather than spike adaptation, whereas in the fast adapting receptor overall adaptation is mainly due to spike adaptation. These conclusions are supported from studies with the slow adapting muscle spindle (Katz, 1950; Lippold, Nicholls and Redfearn, 1960). Since the adaptation of the labellar hairs is slow it is likely that the cause is also due to generator adaptation. It should be emphasized, however, that the work of Nakajima and Onodera (1969a, b) employed constant current injection and precise length-tension stimuli and that until the generator potential of the labellar hair is studied in this way no firm conclusions regarding adaptation can be made.

Dethier (1963) states that the fast adapting tactile receptors are common on areas of the body that contact the environment and that slow adapting receptors are usually proprioceptive in function. The long labellar hairs studied here are an obvious exception to this classification. These hairs are deflected primarily during probing and feeding, the latter lasting up to 10 minutes (Clements, 1963), whereas I have observed probing over an attractive surface lasting up to 30 minutes. It would therefore be of value to the mosquito to have a constant input relating the position of the labella to the substrate. I have demonstrated that the labellar hairs are capable of providing

information to the mosquito about a static deformation but in addition the hairs respond to time-varying phasic stimuli. The latter capability provides information about a constantly changing deflection which is a more realistic system when considering a mosquito feeding substrate such as a flower or animal which are in constant movement.

When random noise is added to the time-varying input a response output results, which is frequency coded, and reliably relates to the phasic analog input. This type of input is likely the most common type of stimulus transmitted from a substrate rather than a pure step deflection or pure sine deflection. The role of neuronal variability in information coding has been discussed by Stein (1970) and Stein and French (1970) and it is not my intention to discuss it here. It has been shown by Stein that an increase in neuronal variability aids in accurate frequency coding of analog input signals. As demonstrated in sec. III-2.22 the addition of random noise to the sine input, which stimulates increase in neuronal variability, eliminates distortion due to rectification at low frequencies and phase locking at higher frequencies resulting in a frequency output which resembles the analog input. In the case of the response of the tactile spine of the cockroach Periplaneta americana located on the leg, the addition of noise to the input increases the linear portion of the response, i.e., the amplitude of the response increases linearly with frequency (Pearson and Holden,

1970). This is seen only at low frequencies for the mosquito labellar hair. As can be seen from the Bode plots for the labellar hair (Fig. 20) the shape of the amplitude and phase curves do not change with the addition of noise except that the low frequency response with the addition of noise does become more linear. Since the low frequency was not extended no actual break frequency was established.

It may be concluded then that the long labellar hairs of A. aegypti respond to both static deflection and to time-varying deflections over a limited frequency range.

V. SUMMARY OF CONCLUSIONS

(1) The external and internal morphological results of the labrum indicates that the apical pegs are basiconic sensilla.

(2) The morphological results of the labellar hairs are inconclusive but the external structure with a socketed hair bound by a membrane indicates that the hairs are sensory.

(3) The apical labral pegs did not respond to any of the stimuli used in this study.

(4) The long labellar hairs do not respond to chemicals but do respond to mechanical stimuli. It is concluded that this mechanoreceptor is responsible for detecting substrate movement, primarily important during feeding.

(5) The results of this study do not reveal the primary receptors responsible for food detection and it is suggested that the cibarial receptors may be important in this respect.

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