Analysis of the Neural Responses of a Cockroach Trochanteral Tactile Hair to Mechanical Stimulation

by

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ABSTRACT

Both adult and juvenile cockroaches of the species Periplaneta americana of both sexes possess prominent hair sensillae on the ventral surface of the proximal segments of all thoracic legs which, when the animal is at rest project perpendicularly downwards from the plane of the animal's body. In this position they would appear to have a possible role as detectors of surface vibration, or irregularities as the animal is moving about. The sensillae could also function as contact chemoreceptors.

This study was undertaken to elucidate the response characteristics of these receptors, with a view to both establishing their function and determining the nature of the receptor mechanisms involved.

Receptor responses resulting from sinusoidal and ramp mechanical displacement stimuli were analysed using a range of techniques, including the averaging of instantaneous rates of receptor firing and cumulative impulse counts using an averaging computor. An exponential regression analysis of the cumulative impulse count of the responses of the receptor to ramp stimulus displacement was carried out using an IBM 3600 computor.

Attempts were also made to record the responses of the hair sensillae in freely walking animals in order to determine their physiological role.

The receptor response to mechanical deflection is a phasic high frequency (700 pps) burst of firing, and the

receptor did not exhibit chemosensory responses. Impulse firing synchrony to the stimulus is maintained at frequencies in excess of 300 cps, and the receptor has a relatively slow time course of habituation.

The number of impulses generated in response to a given velocity mechanical stimulus is a power function of the stimulus displacement, and all receptors obey this function closely ($P > 0.005$).

Unfortunately, the physiological role of the receptor hairs could not be established.

It appears that the method of analysis employed in the study may permit analysis of the different components involved in the receptor responses.

ACKNOWLEDGEMENTS

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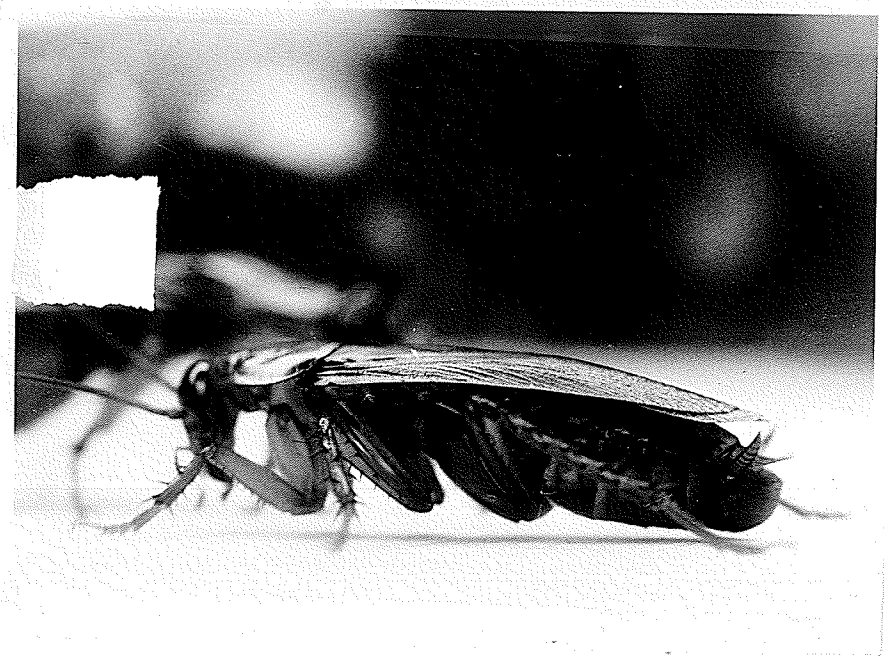
Plate 1. Two postural attitudes commonly assumed by resting cockroaches. (Periplaneta americana)

Top. Low position - in this position the trochanteral and perhaps the femoral hair sensillae are in contact with the substrate, in which position they could act as substrate vibration receptors - this position is often observed in resting healthy animals, and is also a characteristic posture of animals in poor condition.

Bottom. Raised position - this is the more commonly seen resting posture, and the sensillae are lifted well clear of the substrate. When the animal is feeding or moving about they also assume essentially the same posture.



INTRODUCTION

Arthropods differ from most other organisms in having a rigid chitinous exoskeleton, which serves both to protect the animal's internal environment, and as an attachment for the musculature required for movement.

Normally this exocuticle is non-living and rigid, as indeed it must be, and considerable pressures relative to the animal's size are required to deform it.

Since an insect must have information about the nature of the environment in which it exists, there must exist mechanisms whereby external stimuli can be detected and transduced and the information made available to the CNS.

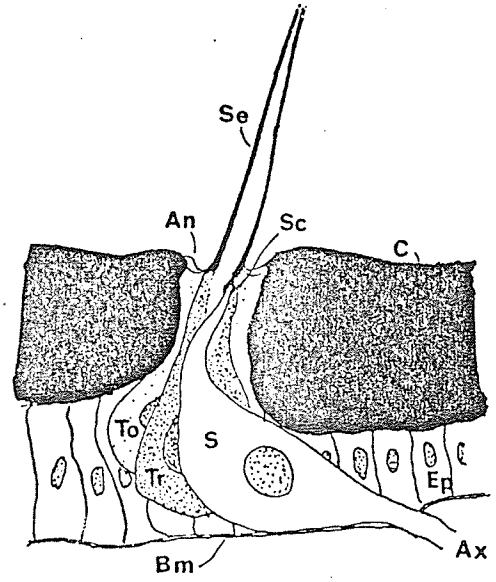
To provide the necessary sensitivity in the presence of the exocuticle, the insects have evolved a vast array of projections and modifications of the exocuticle, which function as transducing windows in the animal's 'shell'. Transducing windows because the projections and modifications usually serve to translate one variable, such as displacement into another, distortion of a surface, and so permit the sensory cells to encode this as a train of impulses.

Such windows are most numerous on the perimeter of the animal, those parts which make the initial contact with the environment - the antennae, head, legs and tip of the abdomen. Large numbers also surround the articulating surfaces of every joint, and these are generally held to be proprioceptors (Dethier 1963).

Basic Structure of Hair Sensillae

Central to all peripheral receptors is a modified cuticular area. This modification may be in the form of a hair or peg (sensilla trichoida), a plate (sensilla placodea), a thin domed region of the cuticle (campaniform sensilla), or an open tipped hair (chemo sensory). It is generally assumed (Snodgrass, 1935) that the archetypal sensilla pattern is that of the hair sensilla, being derived from setae, and that other sensillae are in turn derived from it.

Essentially the hair sensillae consist of a long hollow bristle of varying rigidity inserted into an annulus of membranous exocuticle surrounded by a ring of thick exocuticle. The membranous annular ring functions both as a fulcrum and to provide a restoring force to the sensilla after the disturbance which caused the deflection has passed. In many hair sensillae, the leverage ratio inherent in the structure is such that the neural transducer proper behaves as an isometric force transducer, while the tip of the hair behaves as an isotonic level. The displacement division ratio can be in excess of 200:1 in very long hairs.



An	Annular ring of thin cuticle
Ax	Sensory cell axon
Bm	Basement membrane
C	Cuticle
Ep	Epidermal cells
S	Sensory cell
Sc	Scopopoid sheath
Se	Setae
To	Tormogen or socket cell
Tr	Trichogen or hair forming cell

Diagrammatic anatomy of Sensilla trichoidea (after Snodgrass 1935)

In the simplest sensilla trichoida, only one sensory neurone is found, and such hair receptors are generally simple mechano receptors. However more than one sensory neurone may be present.

In general the sensory neurone, which is considered to be a primary sensory neurone (Dethier 63), is bipolar, the point of insertion of the spical dendrite region being at varying distances up the hollow hair shaft. Associated with the dendrite of the sensory neurone is a tubular and possibly chitinous sheath, the scolops, which appears to be a consistent feature of all sensillae derived from the archetypal trichosensilla (Dethier 63). Associated with the sensory neurone are at least two other cells, a trichogen, or hair forming cell, and a tormogen or socket forming cell.

As previously mentioned the responses spectrum of the receptor can be determined partly by the structure of the 'hair', and partly by the characteristics of the receptor region of the neurone. Thus a number of sensilla trichoida which appear to have essentially the same structure, may have totally different stimulus-response characteristics.

Mechanoreceptive Hair Sensillae

Cahmi (1969) has described a sensory hair of the simple sensilla trichoida type on the head of locust, Schistocerca gregaria. This hair which possessed a single sensory neurone, was found to be a wind velocity receptor; the neurone firing tonically for a sustained deflection of the hair. The receptor

firing frequency was a linear function of the wind velocity. In addition to being a tonic receptor, this receptor showed a high degree of directional sensitivity by virtue of the curvature of the seta and an assymetric compliance of the socket.

In contrast a tarsal tactile hair described by Runion and Usherwood (1968) from the same animal has a similar structure, but the response of the receptor is a phasic burst of impulses following sustained mechanical displacement.

A major problem encountered during the present study has been the dearth of studies on insect mechanoreceptors. Most workers have concentrated on insect chemoreceptors or on the mechano and propioceptors of other arthropod classes, chiefly Crustacea. However the basic mechanisms would appear to be similar (Laverack 1963, 1966; de Forrest Mellon 1963; Wyse 1965; Sanjeeva-Reddy 1971).

In many ways the insect sensilla trichoida mechanoreceptor is analogous to the tylotrich and monotrich sensillae in mammals (Iggo 1968). The hollow setae are analogous to the hair itself, and the point of insertion of the mammalian hair into the epidermis provides both a fulcrum and a restoring force as does the annulus of the tricho sensilla. The basket of free nerve endings which surround the base of the mammalian hair and the dendrite of the tricho sensilla both perform the transduction of the movement into neural impulses. There also appears to be considerable parallels in the diversity of the stimulus response spectrum, and in the presence of multiple

neural endings encoding different parameters of the stimulus.

Both Lowenstein (1958) and Eyzaguirre and Kuffler (1955) have demonstrated the nature of the generator potential in the Pacinian corpuscle and Crustacean stretch receptors respectively. No comparable work appears to have been carried out on mechanoreceptors in insects. However, Wolbarsht (1960) recorded what he referred to as a 'receptor' potentials from the cut end of mechano sensory hairs in a number of insects, in response to mechanical deformation. The magnitude of the receptor voltage recorded ranged from 1 to 10 mV, and was either transient or sustained in response to sustained deformation of the hair, depending on whether the receptor was of a fast or slow adapting type. However the polarity of the receptor potential is negative, in contrast to that observed by intracellular recording. Wolbarsht has concluded that this extracellular recorded potential represents the potential difference between the dendritic and spike generating regions of the receptor neurones. He also concluded that the spike was generated proximally to the receptor site and did not invade the receptor region and also demonstrated that spike frequency was a linear function of receptor potential amplitude.

Chemo Receptors

A number of simple chemo-receptors are anatomically very similar to the tricho-sensilla mechano receptor. In many cases tactile tricho-sensillae also possess one or more supernumerary neurones, the dendrites of which pass through the lumen of the seta to terminate at an opening at the tip.

These have been shown to be chemosensory receptors which are capable of responding to chemical substances ranging from aromatic compounds, amino acids, pherones to water (Dethier 1963).

Response Characteristics

Werner and Mountcastle (1965) demonstrated that the mammalian mechanoreceptive endings described originally by Iggo (Iggo's Corpuscles), exhibited a stimulus-response relationship which was a linear relationship between the log of the response and the log of the stimulus magnitude. In other words the receptor response was a power function of the stimulus magnitude.

Unfortunately very little quantitative work has been carried out on insect mechanoreceptors, and most of that which has been carried out has been of a somewhat cursory nature.

Usherwood (1968) working with a chordotonal propioceptor from the tibia-femur joint of the locust Schistocerca, found that this receptor encodes the tibia-femur angle on either side of a centre point as an increasing firing frequency with a high degree of linearity, but with directional ambiguity. The response was comprised of two components, a phasic component with changing angle, and a slowly adapting component proportional to the final angle.

Unfortunately the structure of the insect chordotonal organs, while considered to be derived from the original tricho sensillum (Snodgrass 1935), is considerably different

from that of the hair receptors, although the basic transduction mechanism is, in all probability, the same.

To date the most thoroughly analysed insect mechanoreceptor is the campaniform organ that underlies the large tibial and femoral spines on the legs of cockroaches. These spines, often incorrectly classified as trichosensillae, are relatively massive (Fig. 1), and hinged at the base in such a way that attempts to move them back forces a projection at the base of the spines against a chitinous stop. As a result the spines act as ratchets, allowing the animal to increase its traction when pushing past obstacles.

A campaniform sensilla situated near the stop is distorted when the spine base bears upon it, and functions as an isometric force transducer (Chapman 1965).

Several workers (Pringle and Wilson 1952; Chapman 1963; and Crowe 1967) have analysed this receptor in great detail. The primary aim of their studies has been to develop a transfer function of the stimulus-response relationship in order to permit the prediction of the receptor's responses to time varying signals of the sort encountered in nature. In fact this preparation has become a proving ground for techniques of linear analysis aimed at deriving mechano receptor transfer functions (Holden 1971). Most of its value in this respect lies in its relatively complex responses to a stimulus, as well as its simplicity and the ease of recording responses.

Crowe has shown that the peak firing frequency of this

receptor is a linear function of tension over the range 0.2 to 2.5 g wt., when determined by using ramp or sinusoidal driving functions. However the slope of the relationship, is to some extent also a function of the velocity of the driving waveform, and the linearity does not hold at low stimulus values.

As yet no worker has attempted to determine the aspects of the receptor responses that are physiologically significant to the animal, a task which could prove to be formidable, since we have no intuitive knowledge of the sensory environment the insect dwells in. When Werner and Mountcastle (1965) attempted to do this for a mammalian touch receptor (Iggo's Corpuscle), they had the assistance of a considerable body of psychophysical theory on sensory discrimination from which to select models that could be tested from the neurophysiological data.

As a result of this lack of theory, many of the characteristics of the receptor responses that have been analysed by various workers may in fact be of little or no consequence to the animal, but however, may provide extremely valuable information about the actual transduction mechanisms involved.

MATERIALS AND METHODS

Materials:

Experimental Animals

Adult cockroaches of the species Periplaneta americana were used for all studies.

The insects were bred in large plastic enclosures on a diet of Purina 'Dog Chow' and green vegetable scraps with adequate water. Tubes of rolled corrugated carboard were used to provide resting sites and prevent overcrowding of the animals.

Cockroaches raised in this manner took from 10 to 16 weeks to reach maturity.

Preparation:

The animals were lightly anaesthetized with CO_2 and a leg was cut off at the junction of the coxa and the thorax using a fine pair of scissors. The tibia was also cut off at its junction with the femur to reduce the size of the preparation and to reduce interference from possible stimulation of the distal segments.

The truncated leg was mounted using wax on a cork platform at the centre of a turn-table in such a manner that the trochanteral hair of interest lay at the centre of the turn-table, a 3" diameter disk of cork covered aluminum, the edge of which was calibrated in degrees. The turn-table permitted the preparation to be rotated with respect to the mechanical

stimulating device.

A silver-silver chloride indifferent electrode was inserted into the cut end of the coxa, and the recording electrode inserted through a fine puncture made in the trochanter, just proximal to the hair receptor. The recording electrode consisted of a length of 25 micron gold wire, which proved to be the most satisfactory material for the purpose, since its almost complete lack of rigidity simplified electrode placement. For recording from the isolated leg preparation the placement of the recording electrode was not critical since there was little spontaneous activity in the leg nerves. Good records (action potentials $>$ 200 μV) were obtained with electrode placement within 0.5 mm of the hair. For the free walking preparation placement was far more critical, as will be discussed later.

Due to the rapidity with which the exuded haemolymph coagulated after puncturing the cuticle, and the softness of the gold wire, it was necessary to insert the recording electrode within 30 seconds of puncturing the cuticle.

The electrodes were connected via shielded wire to a capacitatively coupled FET input pre-amplifier, which in turn was connected to a filter amplifier having variable bandpass characteristics. Most recording was carried out with a total gain of 10,000 and a pass band of 330-10,000 Hz.

The turn-table was mounted on a steel ground plate to which manipulators and stands having magnetic bases could be

attached. The ground plate was mounted on a spring-mounted concrete paving slab to reduce the effect of the floor-borne vibrations which might have otherwise affected the preparation. The entire assembly was mounted within a shielded enclosure.

Stimulus System

In order to characterize the responses to mechanical stimuli of the preparation as fully as possible, a ramp mechanical stimulus, in which the velocity and amount of displacement could be independently varied was used. Such a stimulus permits the separation of the dynamic and static components of the receptor response (Crowe 1967). Working on the assumption that the receptors may be involved in avoiding or sensing mechanical disturbances while the animal is walking, and in view of the upper frequency limitation of the transducer, the range of ramp rates used was restricted to between 1.0 to 10 mm/second.

The electromechanical transducer consisted of a miniature 3" diameter loudspeaker, from which the metal surround and cone had been removed, leaving only the armature coil and suspension intact. A disk of thin mica perforated on the periphery was glued across the centre of the coil and in the centre of the disk a small socket was glued into which various coupling rods could be fitted. For most of the recording a 1" long tapered tungsten needle (actually part of a discarded 'Microtrode' (Transidyne-general) was used as the coupling rod.

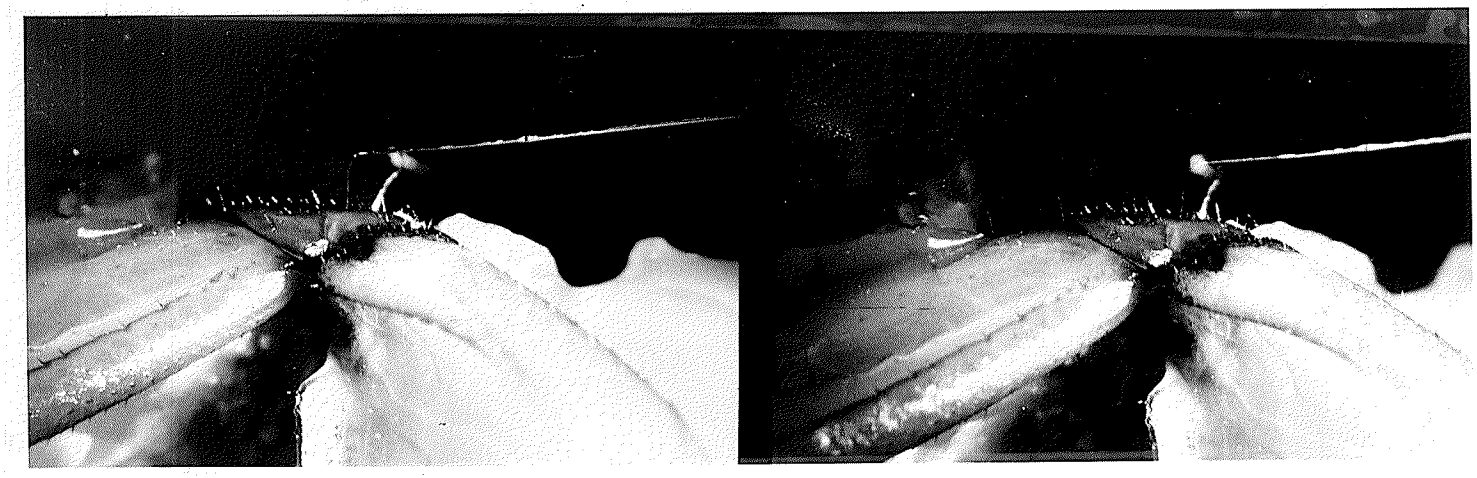


Plate 2. Stereoscopic photograph of a cockroach leg showing the arrangement for stimulating the trochanteral hair sensillum. The tungsten coupling needle can be seen extending from the right of the picture and terminating on the hair. In the background, to the right of the hair, can be seen the 25 micron gold wire recording electrode. This animal has a supernumary sensillum on the left (distal) margin of the trochanter; the coxa is to the right and the femur to the left of the picture.

This picture can be viewed stereoscopically either with a viewer or by holding the picture about 2 feet from the eyes and fusing the images.

The frequency response of this transducer was essentially flat from 0 to 75 cps, after which it peaked (3 x the amplitude at 25 cps) at 175 cps and declined rapidly until at 250 cps, the response was less than 25% of that at 25 cps. Provided the coupling needle was not longer than 1.5", there was little lateral oscillation of the tip within this frequency range.

Within the displacement range ± 0.5 mm, the transducer displacement was a linear function of driving voltage, but became markedly non-linear outside this range. The displacements were measured using a binocular microscope with a calibrated eyepiece micrometer.

To permit tight mechanical coupling between the hair and the transducer, it was necessary to glue the hair tip to the transducer needle with a 'tacki-wax' using a fine (0.1 mm diameter) electrically heated needle. If this was done rapidly enough, no damage to the hair could be observed or detected in the recordings. The use of the wax made it possible to rapidly disconnect the hair from the needle when it was desired to change the direction of stimulation, and to reconnect it again.

Since the maximum displacement of the hair from its resting position without pulling on the hair base was slightly in excess of 0.15 mm, the maximum stimulus excursion was limited to 0.1 mm. One problem associated with the fact that the stimulation needle was rigidly attached to the hair was that there was always some straightening of the normally slightly curved hair with displacement, however,

this was minimized by making the direction of the movement tangential to the arc described by the hair tip (Plate 2).

The ramp generator (Appendix 4) provided a voltage ramp, the slope of which was continuously variable between 0.1 and 10 mm/second, and the height of the ramp could be continuously varied between 0.0 to 0.1 mm, by means of 10 turn potentiometers, which permitted the parameters to be set with an accuracy of better than 1%.

The ramp generator could be triggered by pulses from a time pulse generator, and the duration of the ramp plateau could be varied from 1 to 30 seconds.

The ramp signals were used to drive a linear power amplifier which in turn drove the transducer.

Due to the small displacements involved, and as the hair exhibited very high compliance, the fidelity of the coupled transducer responses to the ramp wave forms was high. Where high frequency sinusoidal stimulation was employed, the use of some sort of servo loop to increase the frequency response would have been beneficial.

Sinusoidal signals were obtained from a gated function generator which generated either bursts of sinusoidal oscillation (1-25 cycles per burst), in response to trigger signals from the timer, or a continuous signal.

Recording Systems

Photographic Records

Initially the action potential records were photographed

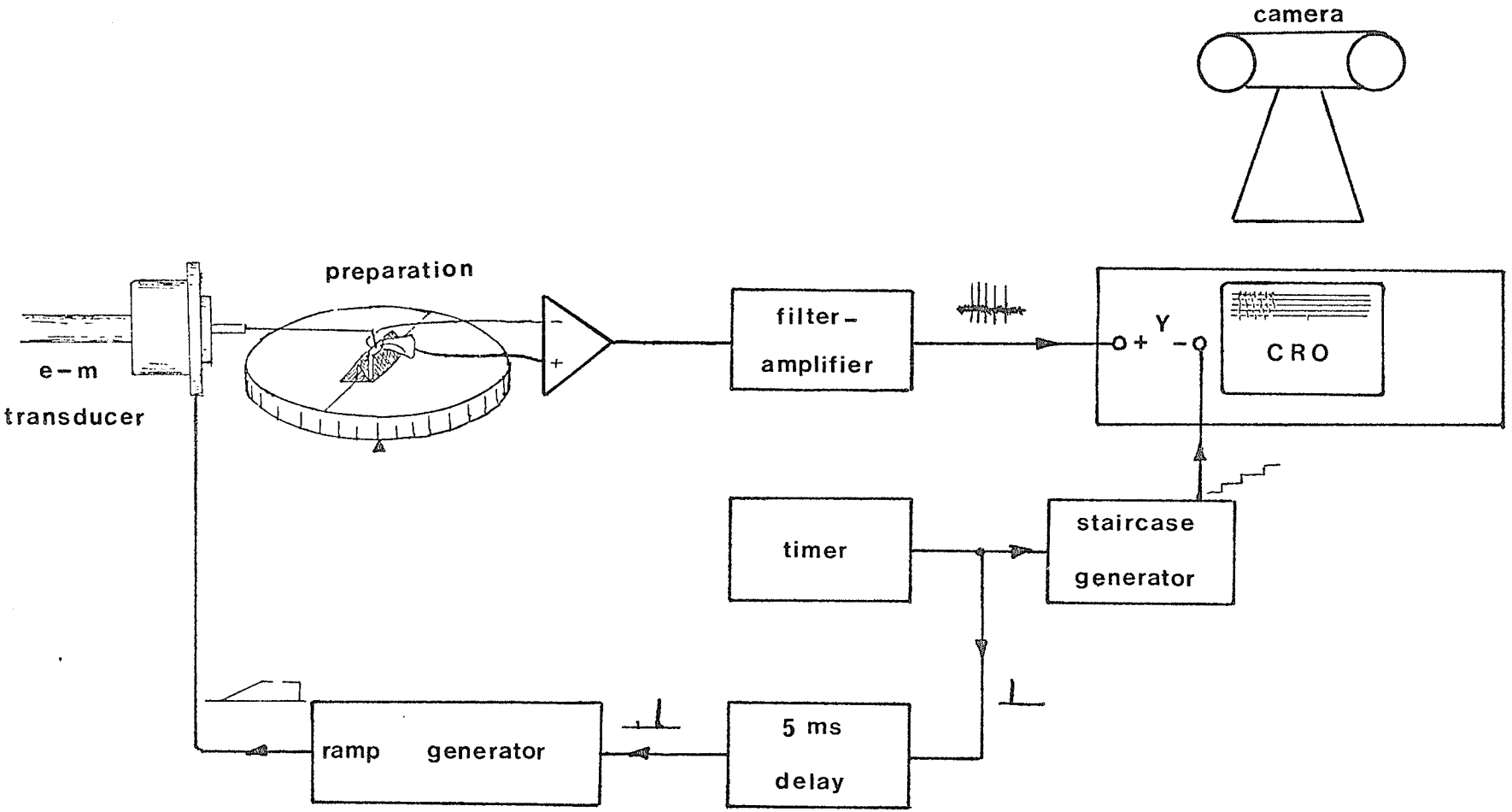

camera
preparation
e-m
transducer
filter-
amplifier
Y
CRO
timer
staircase
generator
ramp generator
5 ms
delay

Figure 1. Initial experimental system used to record the responses to mechanical stimulation on film.

Pulses from the timer oscillator triggered the ramp generator via a 5 millisecond delay circuit, and also triggered the staircase generator. The output from the staircase generator, which serves as a raster generator is applied to the inverting input of the oscilloscope differential amplifier (Y axis), and shifts the trace downwards one increment for each stimulus. The nerve impulses are applied to the non-inverting input of the oscilloscope Y amplifier, which results in each response being recorded sequentially as a separate trace.

The preparation was secured with wax to the triangular cork support on the turn-table, in such a way that the sensillum was perpendicular to the surface of the turn-table. A small micromanipulator fixed to a flexible magnetic stand was used to position and hold the electric-mechanical transducer.

An illuminated Sodeco counter (not shown) positioned in the camera field of view and coupled to the film transport permitted each frame to be identified during later processing.

directly from the oscilloscope screen, using the arrangement illustrated in Fig. 1, and each response individually measured, a procedure which not only was extremely time consuming, but one which prevented the measurement of time intervals to any degree of accuracy. However, this technique, one data frame from which is illustrated in Plate 2, was valuable in that it showed the underlying regularity of the receptor discharge in response to identical stimuli.

The traces were photographed on Panatomic X film using a Cossor 1428 Mk2 oscilloscope camera modified by the addition of automatic single frame advance and an illuminated frame counter, from the face of a 502A (Tektronic) oscilloscope. For some experiments the responses were photographed on moving photosensitive paper (Ilford N.S. 6), the camera being used in the continuous run mode.

Tape Recording

In order to allow fuller use and processing of the data using the CAT 400 averaging computor, it became necessary to record the responses on tape. A single track economy cassette recorder was used and since the speed stability of the tape transport was better than 1% when the recorder was powered from a stabilized power supply, this was adequate for recording the responses from the receptor. Fig. 2 illustrates the set-up employed.

Pulses from the discriminator (which served to extract the action potentials from extraneous noise and background

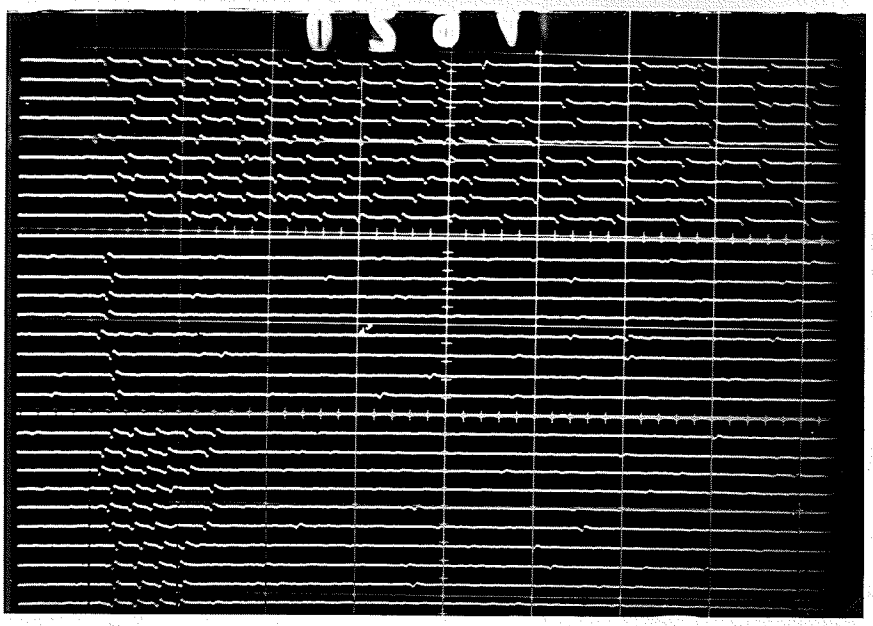


Plate 3. Sample of data frame recorded using the system illustrated in Fig. 1.

The responses illustrated are from preparation B12 - trochanteral hair from the right 3rd thoracic leg of a male cockroach. Sweep speed 10 milliseconds per centimeter. The responses are to ramps of 1.25 mm/second velocity, 0.1 mm displacement (top); 2.5 mm/second, 0.01 mm (middle) and 2.5 mm/second, 0.025 mm displacement (bottom). The regularity of the impulses is clearly evident. A 5 millisecond delay precedes the onset of the stimulus (left side).

Due to the orientation of the counter (top) the inverted numbering was an unavoidable artifact.

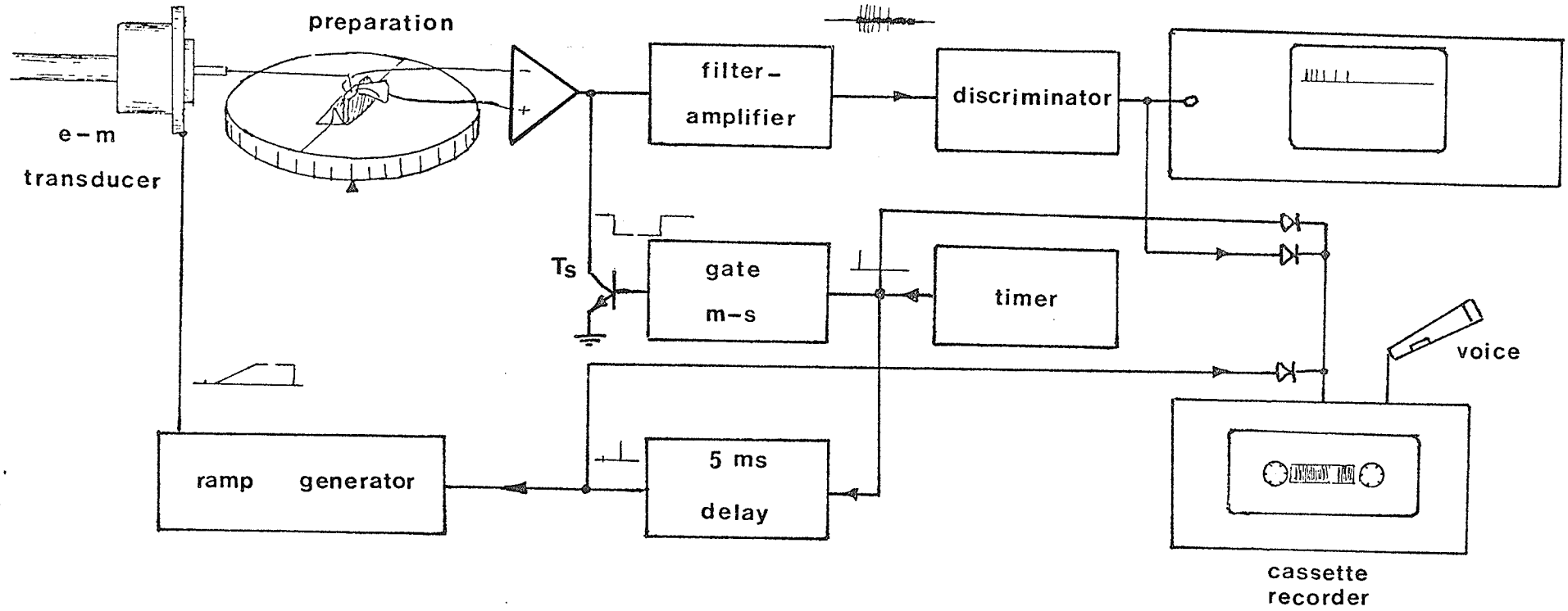

preparation
e – m
transducer
filter –
amplifier
discriminator
Ts
gate
m–s
timer
voice
ramp generator
5 ms
delay
cassette
recorder

Fig. 2. System employed for tape recording the responses to stimulation.

To permit the first pulse of the record (the trigger pulse from the timer) to trigger the CAT 400 sweep circuit in subsequent analysis, since only a one track recorder was available, it was necessary to blank out any extraneous pulses arising from spontaneous activity of other fibres in the preparation during the inter-stimulus interval. This was done by shorting the input to the filter-amplifier to ground by means of a switching transistor. The pulse from the timer triggered the gate monostable circuit which in turn switched off the transistor thus allowing the nerve impulses to be processed for the duration of the gate pulse (120 ms approx.).

Voice commentary was recorded at a low level on the same track, while the pulses were recorded at saturation level.

activity), from the timer and from the 5 ms delay (start of stimulus) were added and recorded at saturation level via the 'Aux' input of the recorder (2v signal for saturation). Voice commentary was also recorded at low levels on the same track.

The addition of the timer pulse and the 5 ms delayed pulse to the record permitted triggering of the oscilloscope or CAT 400 sweep on playback from the first impulse of each record, as well as marking the onset of the stimulus. It also produced a standard 5 ms period which was used to generate calibration points on analysis (Fig. 12).

Since some preparations exhibited some high amplitude background activity which would have caused false triggering of the analysis system, it was necessary to mute the signals from the preparation for the interstimulus interval by shunting these signals to ground with a switching transistor, controlled by a monostable, which in turn was triggered by the timer (Fig. 2). The on period of the monostable controlled the length of time the signals could pass to the amplifier, and was usually 125 ms, the duration of the CAT analysis sweeps used.

Analysis Systems

Instantaneous Rate Analysis

To determine whether there existed a relationship between the initial rate of receptor discharge and stimulus velocity, the recorded output of the receptor was fed via a discriminator

(Fig. 3) which served as a pulse shaper, into an instantaneous rate meter (Appendix 3). This generated on a pulse-by-pulse basis an output voltage which was a linear function of the instantaneous frequency of the preceding pulse pair. The linearity of the rate-meter was better than 2% of output from 70 to 750 pps.

The voltage output from the rate-meter was fed in parallel into the four analogue inputs of a Computor of Average Transients (CAT 400) operating in the averaging mode. Since high temporal resolution was not required the CAT was used in the 4 channel mode (each channel having 100 memory bins), with a sweep duration of 125 msc.

Each group of responses to 10 identical stimuli was routed to each of the CAT inputs sequentially. The averaged responses were plotted out with an X - Y recorder which permitted the responses to four different stimuli to be plotted out at one time, resulting in considerable time savings.

Since the impulses displayed considerable temporal coherence with the stimuli (Plate 3) little temporal resolution was lost as a result of the averaging procedure. As already mentioned, the characteristic of the beat-by-beat instantaneous rate-meter is that the displayed output voltage (i.e., frequency) lags by one pulse interval. While for continuous or smoothly varying discharge rates such as those from mammalian muscle spindles (Harvey and Matthews 1961), this may be of little consequence, for patterned discharge such as those encountered here this lag results in considerable distortion of the

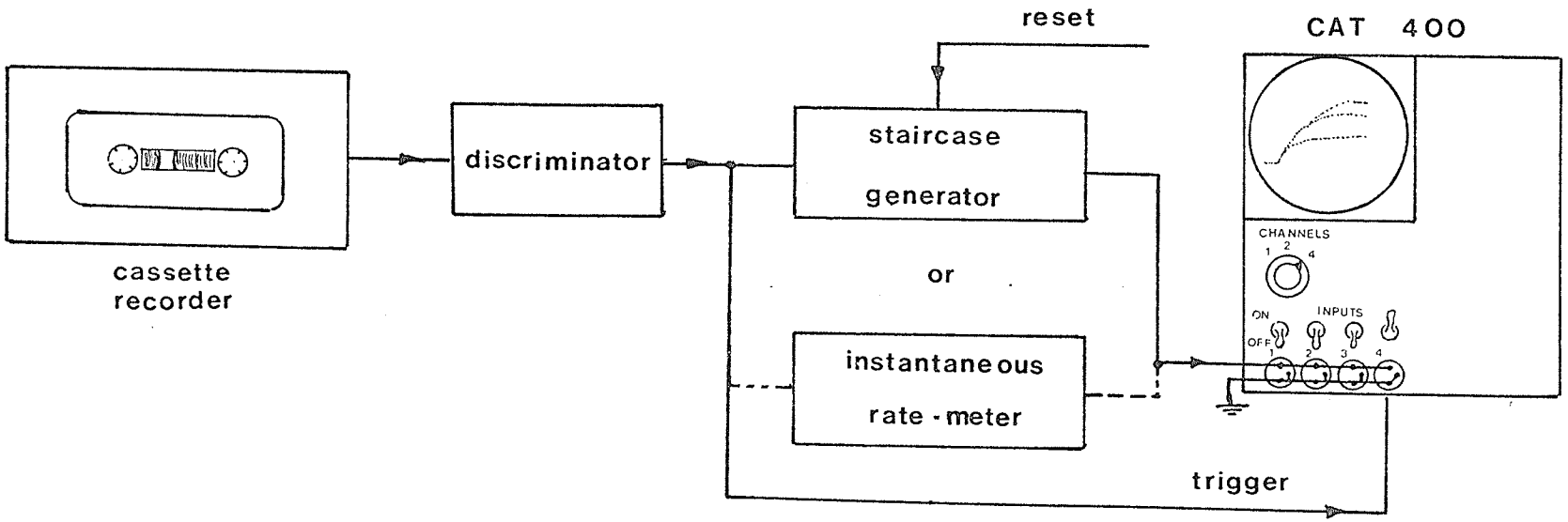

reset
CAT 400
discriminator
staircase
generator
or
instantaneous
rate - meter
cassette
recorder
CHANNELS
1 2 4
ON
OFF
INPUTS
1 2 3 4
trigger

Fig. 3. Analysis system using the C.A.T. 400.

The recorded responses played back from the tape recorder were fed into a discriminator which functioned both as a pulse cleaner and to eliminate interference from the voice signal. The discriminator output pulses were used either to trigger the instantaneous rate-meter or the staircase generator (Cumulative Count Record). The analogue outputs from either of these devices were fed into the analogue inputs of the CAT, the input switches on the CAT being used to feed each separate block of responses to each of the four channels in sequence; the CAT being operated in the 4 channel mode.

Readout of the averaged traces was by way of an X - Y recorder attached to the CAT (not shown).

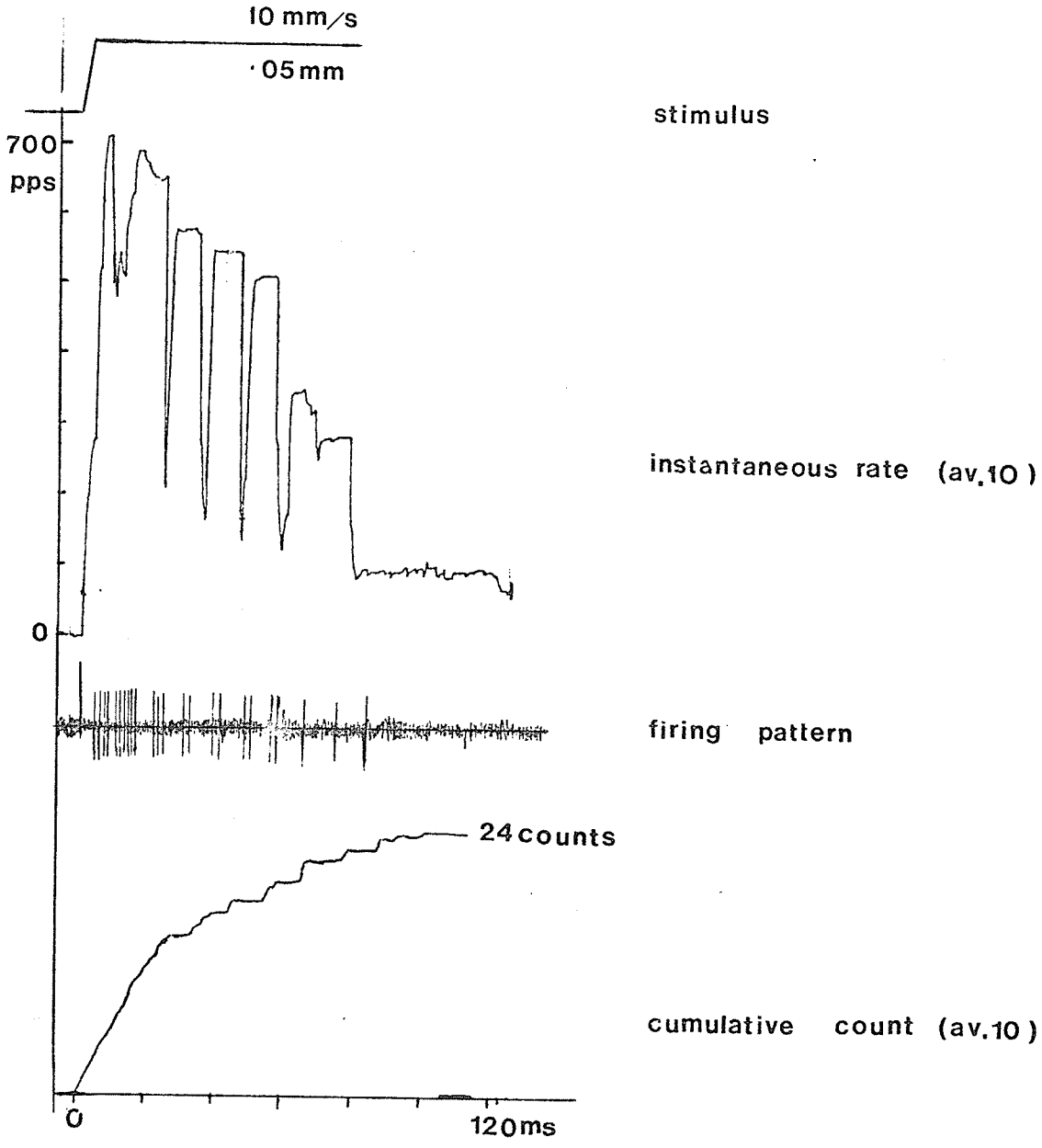

10 mm/s
·05 mm
stimulus
700
pps
0
instantaneous rate (av.10)
firing pattern
24 counts
cumulative count (av.10)
0
120 ms

Fig. 4. Comparison between the stimulus, receptor firing pattern and the outputs of the data analysis methods.

It can be seen that the response duration (at these stimulus parameters) greatly exceeds the stimulus length.

In this preparation (D2-trochanteral hair stimulated at 10 mm/second with a displacement of 0.05 mm), the receptor is exhibiting patterned firing, a response not uncommon at this stimulus velocity. The temporal distortion inherent in the instantaneous rate-meter response is clearly evident, and is due to the fact that the rate-meter output lags by one pulse interval. In addition, the rate-meter output conveys no information about the total number of pulses.

While the cumulative count record obscures the patterned response, it does permit the measurement of the average firing rate from the slope of the curve as well as the number of pulses per response.

Both the instantaneous rate and cumulative count records are averages of 10 consecutive stimulations of the receptor carried out at 2.5 second intervals.

temporal relationship between instantaneous frequency and the pulse interval (Fig. 4). However, for assessing the initial discharge frequency the instantaneous rate-meter is extremely valuable.

The initial (timer) pulse from the tape record served to start the CAT acquisition sweep. The second pulse (stimulus onset) generated a zero rate-meter output for a duration of 5 ms, which permitted alignment of successive plotted traces as well as serving as a zero point.

Averaged Cumulative Count Analysis

Since the number of impulses per stimulus appeared to be the most meaningful measure of the receptor response, the taped responses were processed by averaging the accumulated counts per stimulus for 10 stimuli. This was repeated for each stimulus parameter (Fig. 4).

The shaped impulses from the discriminator are used to trigger the charge monostable of the staircase generator (Appendix 2) and the resulting staircase voltage output was fed into the CAT 400 as described above. The staircase generator was reset either manually or automatically to zero between each sweep.

The resultant plot contains instantaneous rate information (Fig. 4) given by the slope of the curve, as well as the total number of impulses per response.

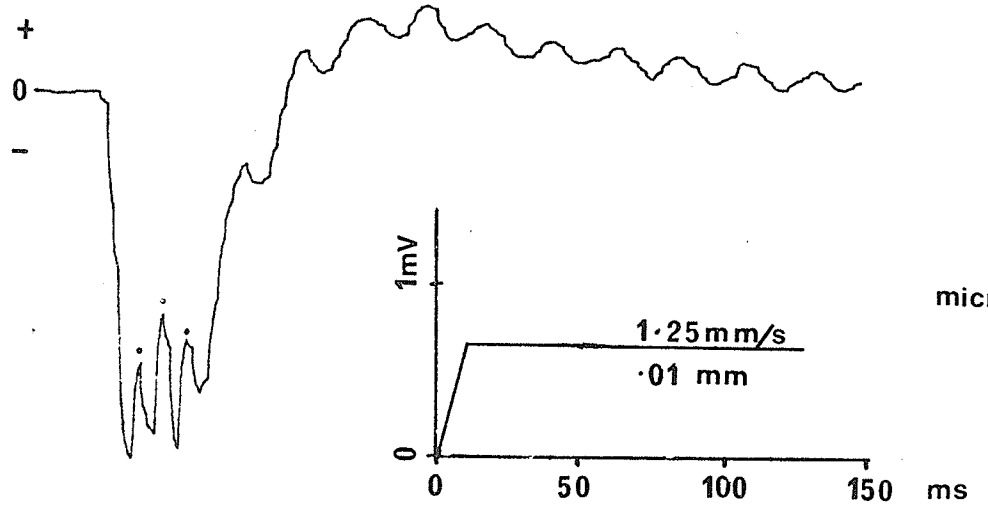
The pen recorder output was so arranged as to plot all the responses to different stimulus displacements at a given

velocity as a family of curves (Fig. 14).

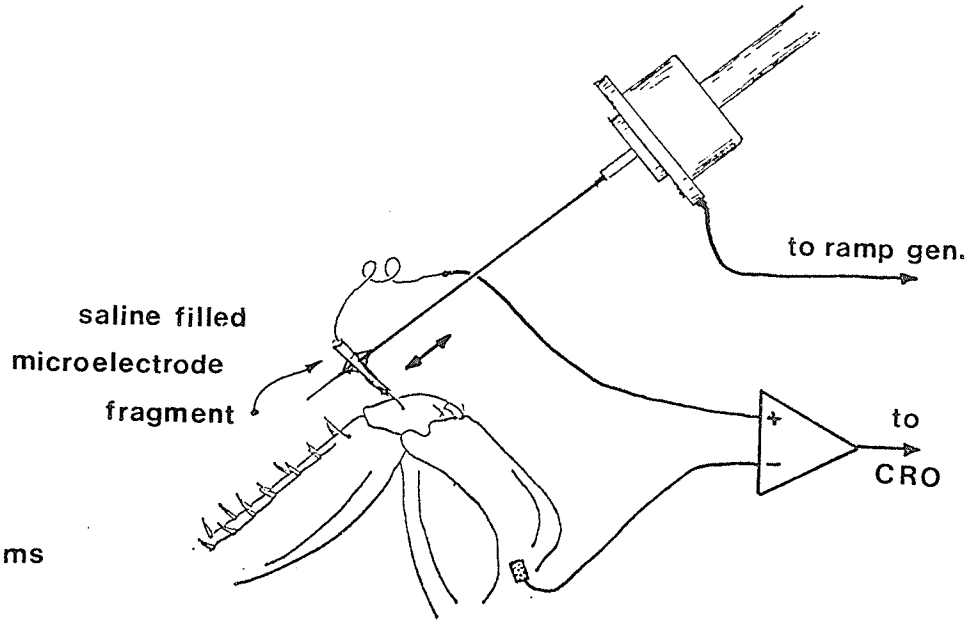
Receptor Potential Recording

In order to record the nature of the receptor mechanism underlying the recorded responses a number of attempts were made to record the receptor potential resulting from a ramp mechanical stimulus using essentially the same method as that employed by Wolbarsht (1960) except that the recording electrode was coupled to the transducer.

A glass micropipette was pulled in the conventional manner from 1.2 diameter pyrex glass and the tip broken back to about 12 micron diameter, so that it would just slip over the cut end of a tactile hair. The electrode was then broken just above the taper, to produce a short (5-7 mm) micro-electrode, which was then filled with saline. The electrode was waxed to the tip of the transducer coupling needle with 'tacki-wax', the wax being melted with an electrically heated hairpin of 38 ga nichrome wire. A fine tinned copper wire (45 micron) was inserted into the shank of the electrode and connected to the non-inverting input of the differential A.C. coupled pre-amplifier. The silver-silver chloride indifferent electrode was inserted in the coxal muscles as described earlier, and connected to the inverting input. Since the input time constant of the pre-amplifier was in excess of 10 seconds, and since the response was a phasic one, little distortion of the recorded waveform occurred as the remainder of the circuits were D.C. coupled.


+
0
−
1mV
0
1·25mm/s
·01 mm
0
50
100
150
ms

A


saline filled
microelectrode
fragment
to ramp gen.
to
CRO

B

Fig. 5

A. Receptor potential from a trochanteral sensillum as a result of mechanical stimulation of the hair shaft by a ramp stimulus. This waveform is the average of 10 responses averaged using the CAT 400. The positive going spikes capped with a dot are action potentials (much attenuated by passage through the hair cell soma). The sinusoidal oscillations are averaged A.C. 50 Hz interference which happened to be synchronous with the timing oscillator. It can be seen that the response is of longer duration than the stimulus ramp, the slight positivity at the end of the response may be an artifact due to the fact that the amplifier was A.C. coupled (Fl 0.1 Hz).

B. Experimental setup used to record the receptor potentials. The saline filled glass microelectrode fragment (about 7 mm long and 50 microns wide at the tip) was secured to the transducer needle with wax, which permitted simultaneous recording and stimulating of the hair receptor and the sensillum was cut about one third the way up. The electrode was slipped over the cut end. Fine 50 micron tinned copper wire coupled the electrode to the recording amplifier, and indifferent Ag/AgCl electrode placed on the cut end of the coxa.

The tactile hair was cut off at about 1/3 its height, and the saline filled microelectrode manoeuvered over it (Fig. 5).

The responses to ramp stimulation were photographed on moving film together with the stimulus (Fig. 20) and in one instance averaged using the CAT 400 (Fig. 5).

Free Walking Preparation

Two insulated 50 micron wires were used both as the electrodes and as the connecting wire to the amplifier, the wires being twisted together. The points of the wire which were to be inserted into the animal were tinned with solder in the conventional manner, and as part of the copper dissolved in the molten solder during the tinning process, the tips of wires became tapered points, which greatly facilitated insertion.

With the animal under CO_2 anaesthesia and prior to immobilizing the animal on its back, the tinned and twisted signal wires were drawn under the left elytra and pulled under the wing base so that the tinned tips of the wires lay about half way along the abdomen. The trailing wire was attached to the pronotum of the animal by means of several blobs of 'tacki-wax', in order to provide strain relief. The animal was then immobilized on its back.

A hole was punched in the trochanter, about 0.2 mm proximal to the sensilla and offset to the ventral side slightly. The recording electrode was bent at 90° about 0.25 mm from the

tip, and inserted into the hole. The wire was anchored by means of small dots of tacki-wax, applied to the cuticle with an electrically heated needle, care being taken not to overheat the cuticle (which often caused injury discharges in peripheral nerves).

The wire was then looped under the trochanter and fastened to the inside surface of the coxa with wax, leaving a loop of wire at each joint to prevent restricting the leg movement.

The other electrode was pushed into the superficial musculature of the abdomen and similarly fastened with wax.

It was usually necessary to perform the entire operation under a stream of CO_2 since the effects of this anaesthesia wore off very rapidly.

Upon complete recovery from the effects of the procedure (usually 30 minutes), the animal showed few apparent ill effects and made the usual escape attempts which are normal to a cockroach transferred to a closed container.

Unfortunately, due to the oily cuticle, this activity occasionally dislodged both the wax anchors and the electrodes. Electrodes secured with 'tacki-wax' were superior to those attached in paraffin beeswax (50:50) in this respect.

In addition, the motor activity associated with the escape attempts masked any responses from the sensilla, and no recording was carried out until the animals became quiet.

Careful placing of the recording electrodes was essential in order to get satisfactorily large potentials to permit the

response to be distinguished from the background noise. Only about half of the preparations were satisfactory in this respect. While the electrode was being inserted, the hair was touched with a fine (00) paint brush, and the position of the electrode tip moved to obtain an optimum response, which was never greater than 300 uV, possibly reflecting the diameter of the axon.

Of the 9 preparations tried, only 2 were really successful. The electrode wires were attached to the differential input of the AC coupled FET pre-amplifier, and both the amplifier and the beaker constraining the cockroach rested on a steel ground plate within a shielded cage. This arrangement did not suffer from hum artefacts, despite the fact that the unshielded trailing wires were over 12" long.

The pre-amplifier output was fed into the filter amplifier, the band width of which was set at 330 Hz - 10 KHz; the total gain being 10,000. The amplifier output was monitored on the oscilloscope and recorded directly on the cassette recorder for later replay and photographic recording (Fig. 23,24).

Chemical Stimulation

Since the position of the hairs on the animal would make them possible contact or surface chemoceptors, this possibility was checked by applying various chemical solutions to the tip of the hair preparation described initially, using either a fine paint brush dipped in the fluid, or a capillary tube filled with the test substances. Since no responses were evoked to any substance, the experiment was not pursued. The

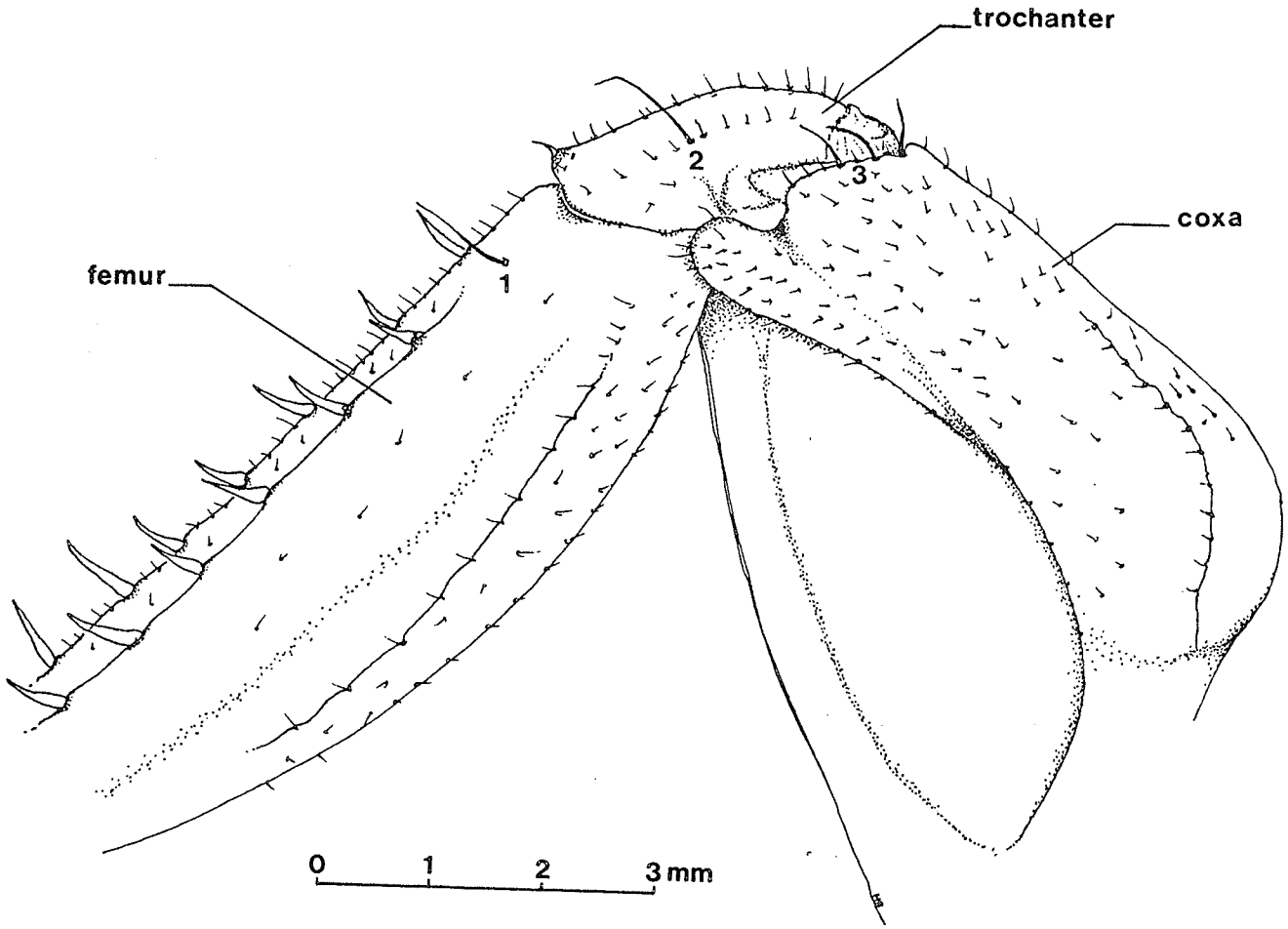

trochanter
coxa
femur
1
2
3
0
1
2
3 mm

Fig. 6. Ventral view of the proximal part of the right third thoracic leg, showing the position and relative sizes of the trochanteral (2) and femoral (1) tactile hairs involved in the study. The coxal (3) hairs were not investigated. Most of the smaller cuticular hairs did not appear to generate a neural response to mechanical stimulation. Legs on both sides of the animal showed essentially the same distribution of the major sensillae, although supernumerary hairs appeared in some animals (see Plate 2).

The large spines on the anterior margin of the femur are similar to those studied by Chapman (1963,1965) and Crowe (1967).

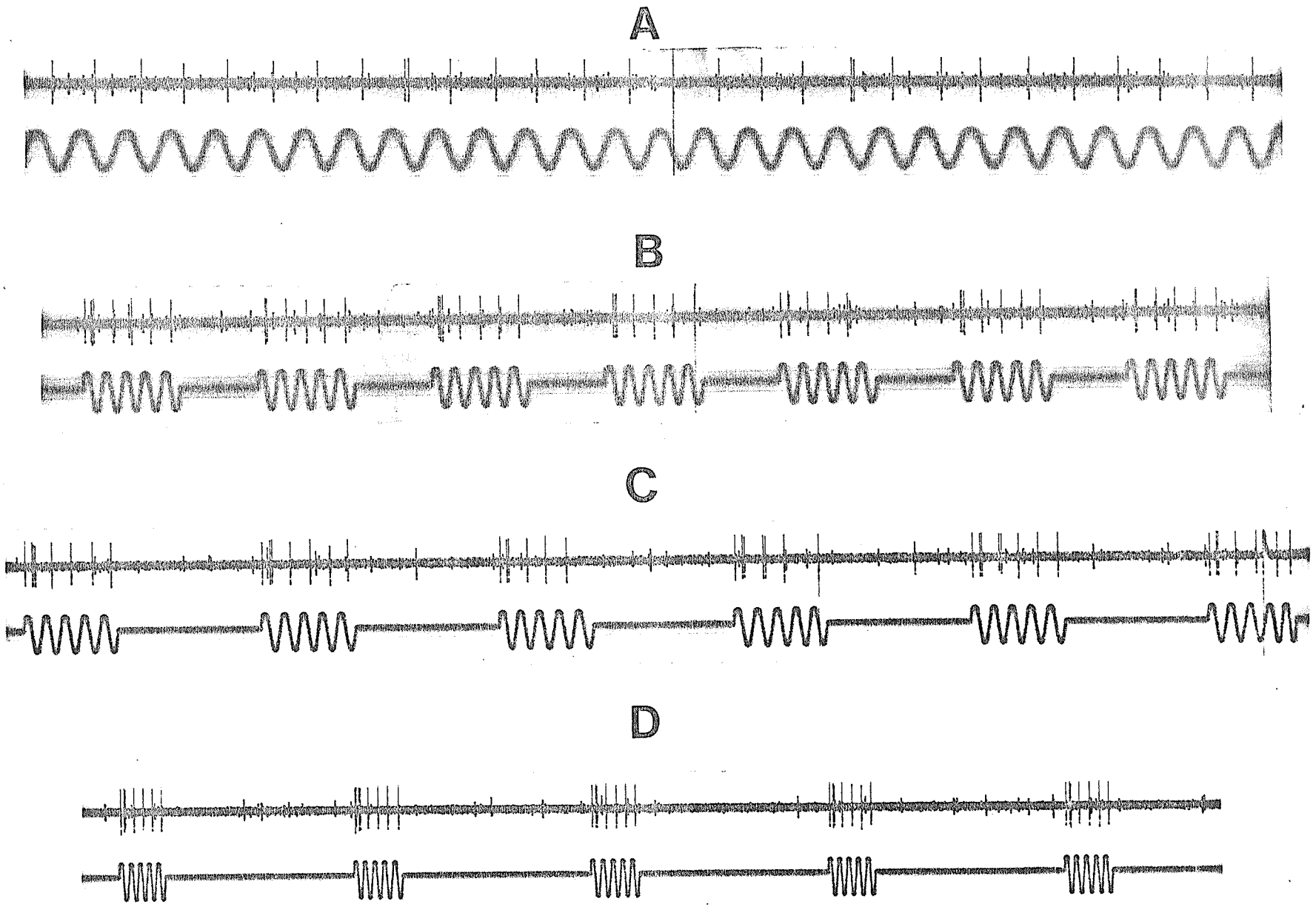

A
B
C
D

Fig. 7. Responses of the trochanteral hair sensilla to bursts of sinusoidal mechanical stimulation. The amplitude of the stimulation was kept constant throughout and above threshold; $\pm$ 0.01 mm. It can be seen that in general the receptor fires once for each cycle of stimulation and shows a burst of impulses at the start of each stimulus burst and then which rapidly adapts.

Stimulus frequencies A: 10 cps, B and C: 25 cps, D: 50 cps.

test substances that were tried were water, pH 2, 6, 12 buffer solutions, N sucrose, normal saline and 50% alcohol.

Distribution of the Sensillae

The sensillae occur on the ventral surface of the trochanter and femur of all 3 pairs of legs, and project downwards approximately perpendicular to the longitudinal axis of the insect's body. In this position, the sensillae can either brush or rest against the substrate when the animal assumes a suitable posture (Plate 1).

One sensillum occurs prominently on the trochanter and one on the upper femur (Fig. 6), while on the coxal-trochanteral joint three or four somewhat smaller sensillae fringe the margin of the socket. These last sensillae are not as prominent and do not project out in the manner of the trochanteral and femoral hairs, and were not considered in this study.

The position and distribution of the sensillae varied little from animal to animal and from one to another of the six legs. However, some animals possessed supernumerary hairs near the distal margin of the trochanter and on the femur (one such is visible in Plate 6).

Anatomy of the Sensillae

The sensillae exhibit the basic structure of trichosensillae as described by Snodgrass (1934), Dethier (1963) and others. It consists of a long, hollow straight or slightly curved setae, about 1-2 mm long, which tapers from 11 μ at the base to 2 μ at the tip. The base of the setae is inserted into an annular ring of thin cuticle, which is

surrounded by a raised thickened cuticular annulus.

Unfortunately, it has not been possible to perform histological studies to determine the presence and distribution of sensory neurones.

In whole mount preparations stained with methylene blue using the method described by Laverack for Crustacea (Laverack 1964), little detail of nervous structure could be made out. Laverack was unable to use the technique to demonstrate sensory neurones in scorpions, and suggested that the failure could be due to the thickness of the peri-neural sheath (Laverack 1966). The fact that insects also possess a thick neural sheath could explain the present failure.

In examining whole mount preparations in which the methylene blue staining procedure had been attempted, there did appear to be a point on the bases of all the receptor hairs examined to which the dendrites of the sense cell could have been attached. If this were so, the structure of the sensillae would have agreed with that described by Snodgrass for the sensilla trichoidea (Snodgrass 1935).

The presence of a tonic response in some sensillae would argue for the presence of at least 2 sensory neurones at the receptor base.

Although not directly observed, the sensory axon from the receptor joins the large mixed nerve, nerve 5 (Nijenhuis and Dresden 1954), which runs the length of the leg and enters the posterior margin of the thoracic ganglion of each segment.

RESULTS

Response to Sinusoidal Mechanical Stimulation

The threshold for stimulation varied with different preparations and with the rate of displacement, but in general, tended to be about 0.004 mm as determined from ramp stimuli.

At stimulus amplitudes just above threshold, the receptor would fire coherently once with each cycle of stimulation for frequencies exceeding 300 cps. However, it was not feasible to measure the absolute changes in effective stimulus amplitude with increasing frequency because of the cut off frequency of the transducer and lack of suitable apparatus to measure small deviation at high frequencies. The receptor frequency response could have been measured with a piezo electric bimorph 'bender' of the type employed by Sato (1961) to stimulate the pacinian corpuscle provided it had sufficient displacement range. Since the transducer output displacement falls rapidly with increasing frequency beyond 170 cps, it is probable that the threshold at high frequencies may be lower than for frequencies of 100 cps or less. Similar behaviour has been reported by Usherwood for the tarsal hair receptor of the locust (Runion and Usherwood 1968), and by Pringle and Wilson (1951).

For sinusoidal stimuli above threshold amplitude the receptor produces multiple bursts of spikes for each stimulus

cycle. The receptor then rapidly adapts within the period of two or three stimulus cycles and the response remains constant at one spike per cycle (Figs. 7, 8).

Responses to stimuli bordering the threshold value (Fig. 8) remain coherent with the stimulus, but do not necessarily occur at the onset of stimulation. This behaviour is probably due to the summation of the sub-threshold receptor potential resulting from the stimulus, and internal 'noise' in the receptor itself. Where the sum exceeds threshold, spikes occur.

Not all sensillae tested appeared to be able to respond to sinusoidal frequencies in excess of 100 cps, but no attempt was made to determine the proportion of insensitive sensillae. However, it would appear that more than 70% of the sensillae can respond to frequencies well in excess of 100 cps. This contrasts with the behaviour of the locust tarsal hair sensillae described by Usherwood (Runion and Usherwood 1968), which did not respond synchronously to sinusoidal stimuli with frequencies greater than 100 cps.

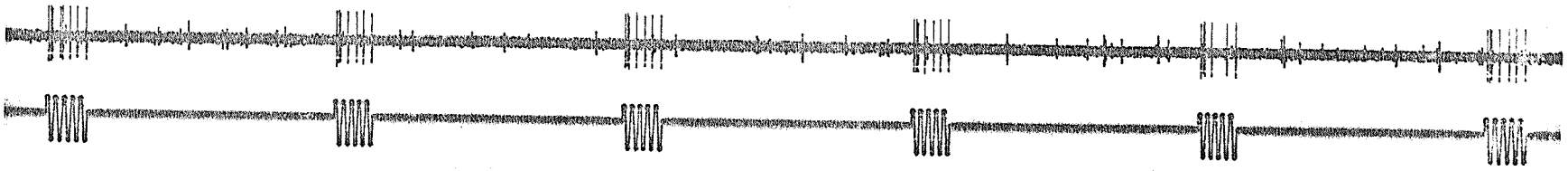
One receptor (Fig. 9) exhibited synchronous after discharges to bursts of sinusoidal stimulation at frequencies in excess of 100 cps. At 100 cps (A in Fig. 9), the stimulus was above threshold for this preparation and in the response illustrated, the receptor was firing twice per stimulus cycle. At the end of the stimulus burst, the receptor continued firing at the stimulus frequency for 1 or 2 more spikes. At higher frequencies, the number of after discharge spikes

increased to 6 at 300 cps. While the spikes retained coherence with the signal at 300 cps, the after discharge firing declined in frequency in steps that were sub-multiples of the stimulus frequency. This response of the hair receptor may possibly have been due to 'ringing' of the stimulator or its mountings at this frequency, but since the mass of the transducer armature assembly is small (about 5 gm) and since the support was a massive magnetic base stand, this is unlikely.

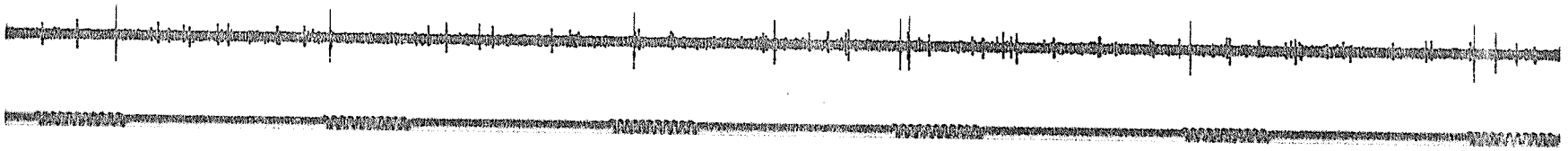
Those receptors responding to stimulation at frequencies in excess of 100 cps also show low rates of habituation to repetitive sinusoidal stimuli. Figs 10A and B show the nature of the responses of a trochanteral hair sensilla to sinusoidal mechanical stimulation, the frequency of which has been varied from 25 to 120 cps and back. While the receptor fires repetitively at the low frequency stimulus, at increasing frequencies there is a 1:1 stimulus response relationship which shows little habituation, partly due to the increasing transducer displacement as the transducer approaches its peak frequency. In the record shown in Fig. 10A, the receptor can be seen to fire twice per cycle at the peak of the stimulus amplitude. The sporadic but synchronous firing as the amplitude of the stimulus decreased demonstrates the presence of a raised threshold.

To characterize the degree of habituation shown by the receptor, it was subjected a continuous sinusoidal mechanical stimulus above threshold for the preparation (0.04 mm) for

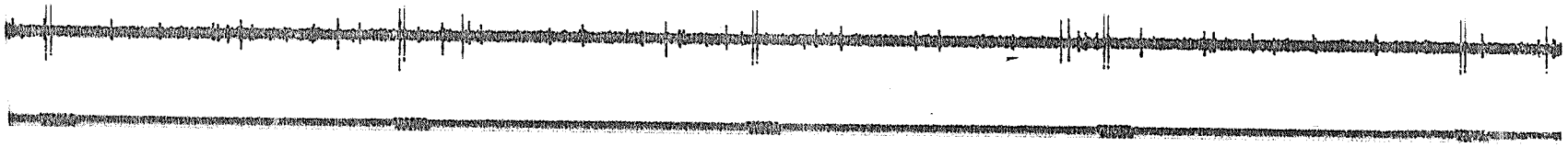
E



F



G



H

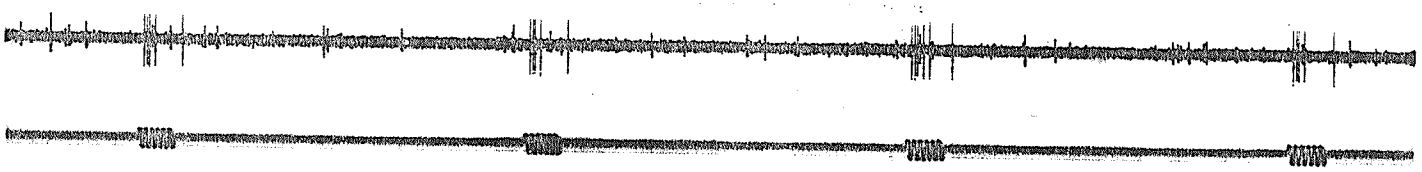


Fig. 8. Responses of the trochanteral hair to bursts of sinusoidal mechanical stimulation. Same preparations as in Fig. 7.

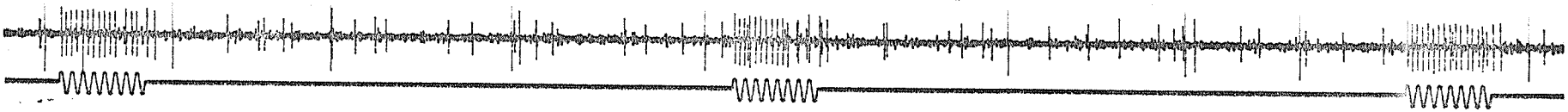
E. 75 cps, amplitude above threshold for this preparation, ± 0.01 mm.

F. Threshold response at 75 cps, stimulus amplitude ± 0.0025 mm (the gain of the lower trace is unchanged). The time of occurrence of the response within each stimulus burst tended to be erratic but synchronous, a response characteristic of threshold responses.

G. Threshold response at 100 cps (± 0.002 mm).

H. Response at 100 cps at increased amplitude (± 0.004 mm).

A



B

C

Fig. 9. After discharges following sinusoidal stimulation at high frequencies. The amplitude at 100 cps (± 0.013 mm) was about twice threshold for this preparation.

A: 100 cps, B: 200 cps, C: 300 cps. It can be seen that the receptor responds at all frequencies in synchrony with the stimulus, the response at 100 cps showing doubling of the number of spikes. Despite the constancy of the stimulus amplitude in the lower trace of each trace, the amplitude of the actual mechanical stimulus declines with increasing frequency because of the limited frequency response of the transducer (see text). As a result of this, the stimulus amplitude at 100 cps, ± 0.013 mm declines by about 2/3 at 200 cps, and to about 1/8 at 300 cps.

It can be seen that at all frequencies, and especially at 300 cps, there is a prolonged after discharge which maintains synchrony with the original stimulus. This discharge is not due to oscillation of the relatively well damped transducer or its mountings.

The dots in the bottom trace mark the after discharge spikes, and it can be seen that the interspike interval increases by regular multiples of the original period.

A

250ms

B

100ms

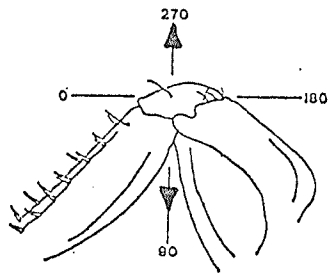


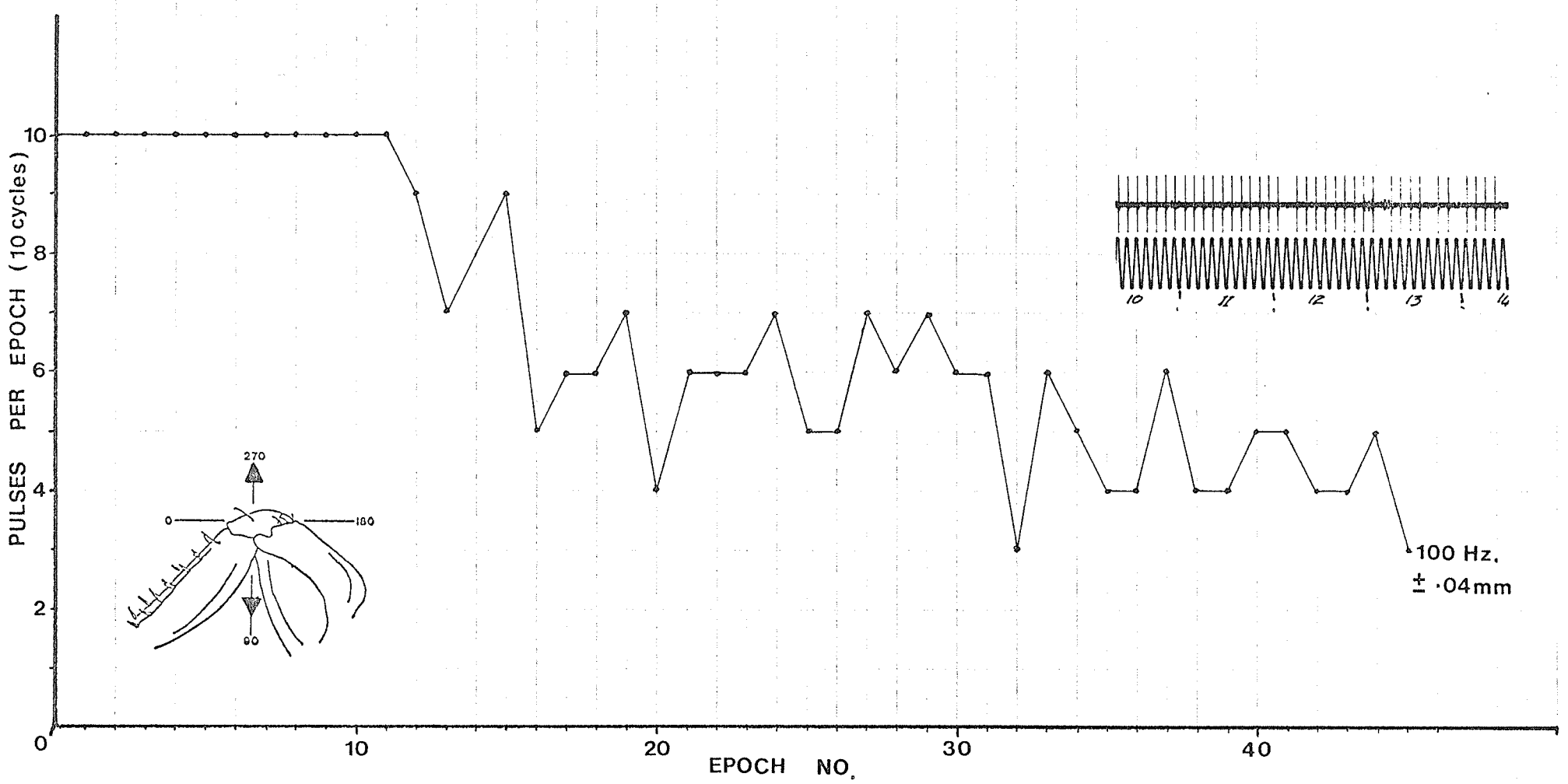
Fig. 10. Response of trochanteral hair receptor to mechanical stimulation with a suprathreshold frequency swept sinusoidal stimulus. Orientation of the plane of the stimulation is given by the inset diagram. Frequency sweep is from 25 to 120 cps in a non-linear manner.

A. Entire record for one sweep.

B. Expanded portion of a second record.

In this pair of records, the stimulus signal was taken from the coil of the transducer, and the increase in signal size at high frequencies reflects the peaking of the transducer response at this frequency.

Fig. 11. Time course of habituation of a trochanteral hair (D3) subjected to a 100 Hz $\pm$ 0.04 mm sinusoidal movement (just above threshold for the receptor). At this level, the receptor fired once per cycle for 110 cycles after which the proportion of dropped responses increased slowly. A slight increase in stimulus strength to $\pm$ 0.06 mm restored the response to the original level (not shown). The film record inset is from the 10th to the 14th epoch (1 to 1.4 seconds after onset).


PULSES PER EPOCH (10 cycles)
10
8
6
4
2
0
10
20
30
40
EPOCH NO.
270
0
180
90
10
11
12
13
14
100 Hz.
± ·04mm

30 seconds, and the responses recorded on moving film. From the film record a plot was made of the number of pulses per 10 cycle epoch against time. It can be seen that habituation developed only after the first second and progressed slowly until by 4 seconds it was firing in response to about 50% of the stimuli. Increasing the stimulus displacement to ± 0.06 mm restored the response to its original 1:1 relationship, showing that the drop in response was due to habituation and not fatigue.

Responses to Ramp Stimulation

Qualitative

Initially the sensitivity of the receptors investigated was found to be low and erratic, but various improvements in technique, specifically the use of the harder tacki-wax to cement the hair tip to the transducer rod; the use of electrodes of inert material (Au and Ag/AgCl) rather than the copper wire used by various authors (Hoyle 1964), and the control of evaporation from the cut coxal surface improved the responses considerably, and later preparations tended to be relatively uniform in their response characteristics.

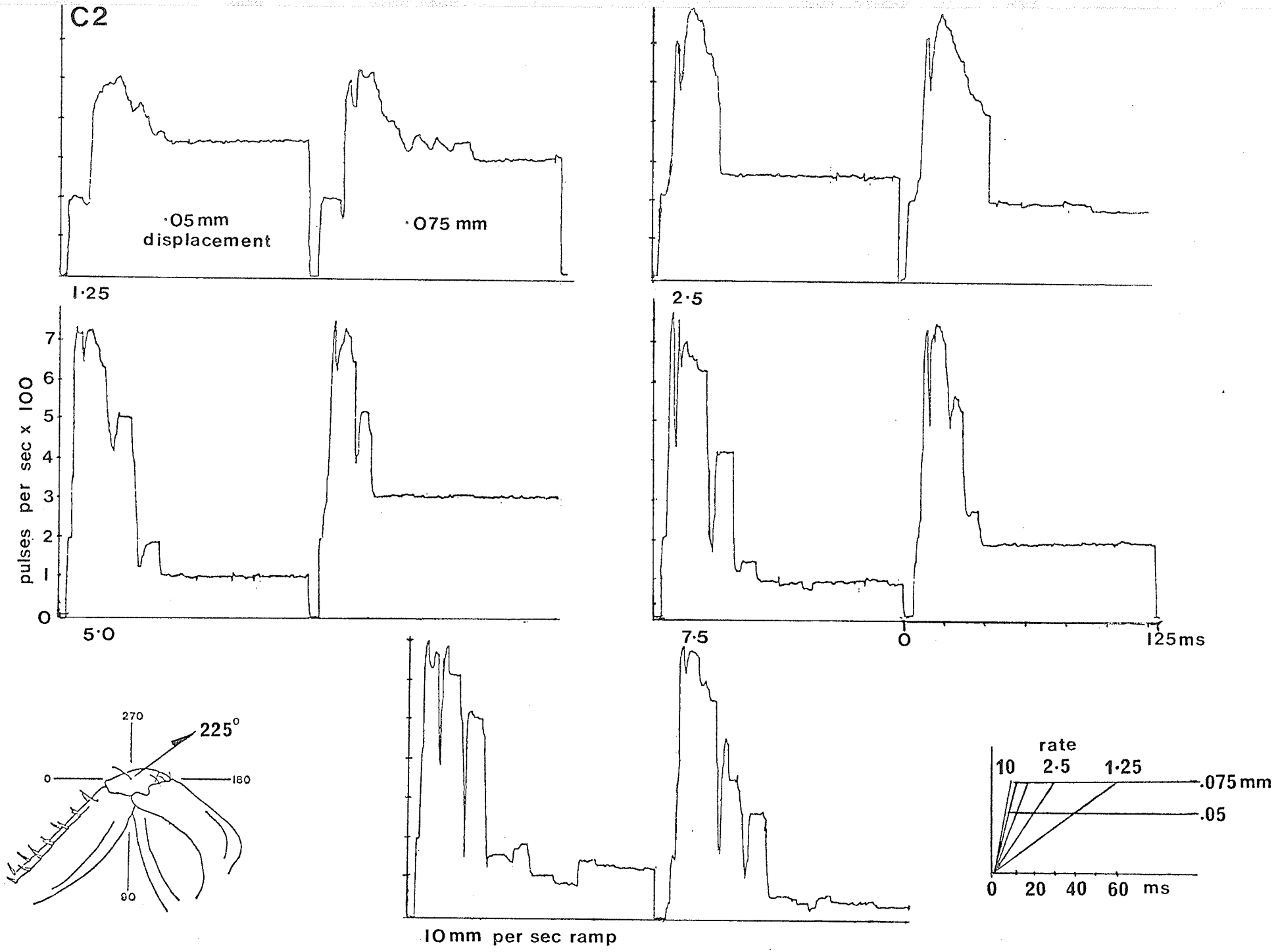
In all, about 45 trochanteral hair sensillae preparations were used, and of these about 30 yielded satisfactory records in that the preparations did not suffer from excessive background activity, and that they maintained a consistent level of responsiveness for at least one complete series of stimulus presentations.

Fig. 12. Averaged instantaneous firing rates for a trochanteral hair (C2) for different stimulus velocities and displacements. Each histogram is the average of 10 responses, and the stimulus orientation is 225° to the axis of the trochanter (see inset diagram).

It can be seen that the initial firing rate rapidly reaches maximum (750 pps) with increasing ramp velocity. The curves on the RHS can be used to determine stimulus duration for various velocities and displacements.

There are a pair of histograms for each stimulus velocity, the right hand and left hand histogram are for displacements of 0.05 and 0.075 mm, respectively.

The time base (0 - 125 mS) and impulse frequency calibration scales apply to all the histograms.


C2
·05 mm
displacement
·075 mm
1·25
2·5
pulses per sec x 100
0
1
2
3
4
5
6
7
5·0
7·5
0
125ms
270
225°
0
180
90
10 mm per sec ramp
rate
10
2·5
1·25
.075 mm
.05
0
20
40
60
ms

Quantitative Stimulus Velocity

All the trochanteral hair sensillae investigated exhibited an increase in initial firing rate with increasing stimulus displacement velocity (= ramp slope), provided the displacement is adequate. As can be seen from Figs. 12 and 13, the increase would appear to plateau rapidly to a maximum firing rate of 600 to 750 pps. However, before the plateau velocity is reached, the initial firing frequency appears to be a linear function of stimulus velocity. In other words, the firing frequency F is proportional to the derivative of the stimulus velocity.

$$F = K_1 \frac{dS}{dT} \quad \ldots\ldots\ldots\ldots 1$$

where K_1 is a constant.

In this respect, the hair receptor behaviour is similar to that of the primary ending of the mammalian spindle (Harvey and Matthews 1961).

At stimulus velocities in excess of that at which the firing frequency peaks (about 5 mm/s), the firing rate declines somewhat, possibly due to receptor fatigue.

Some directional sensitivity in this response is evident both for the receptor illustrated in Fig.13 and in others investigated, but this directionality does not appear to follow any consistent pattern, and may merely reflect underlying structural assymetries in the sensillae.

The effect of increasing stimulus velocity on the number of pulses per response to a given displacement, is to cause saturation of the response. This is clearly evident in the

responses illustrated in Fig. 14. Despite the compression of the responses, the receptor appears to obey the same non-linear relationship between displacement and response. This will be discussed in full later.

One prominent characteristic of the receptor's response to identical stimuli is its extreme regularity as can be judged from the responses shown in Plate 3, and from the patterning evident in the instantaneous rate histograms, despite the fact that they represent the average of 10 responses.

Such regularity would appear to be due to the existence of an underlying rhythmic mechanism set into action by the non-rhythmic receptor potential as suggested by Schwartzkopff (1964).

Further evidence for this rhythmicity could be deduced from the occurrence of patterning of responses often seen when a receptor is given ramp stimuli having high velocities and large displacements. This patterning manifests itself as regular caps in the pulse train, as shown in the responses illustrated in Figs. 4 and 12.

Stimulus Displacement

The height of the displacement stimulus has no effect on firing rate per se but only on the number of impulses generated per response (Plate 3). The possibility that the receptor encodes displacement by the number of impulses per burst was tested by recording the cumulative impulse counts

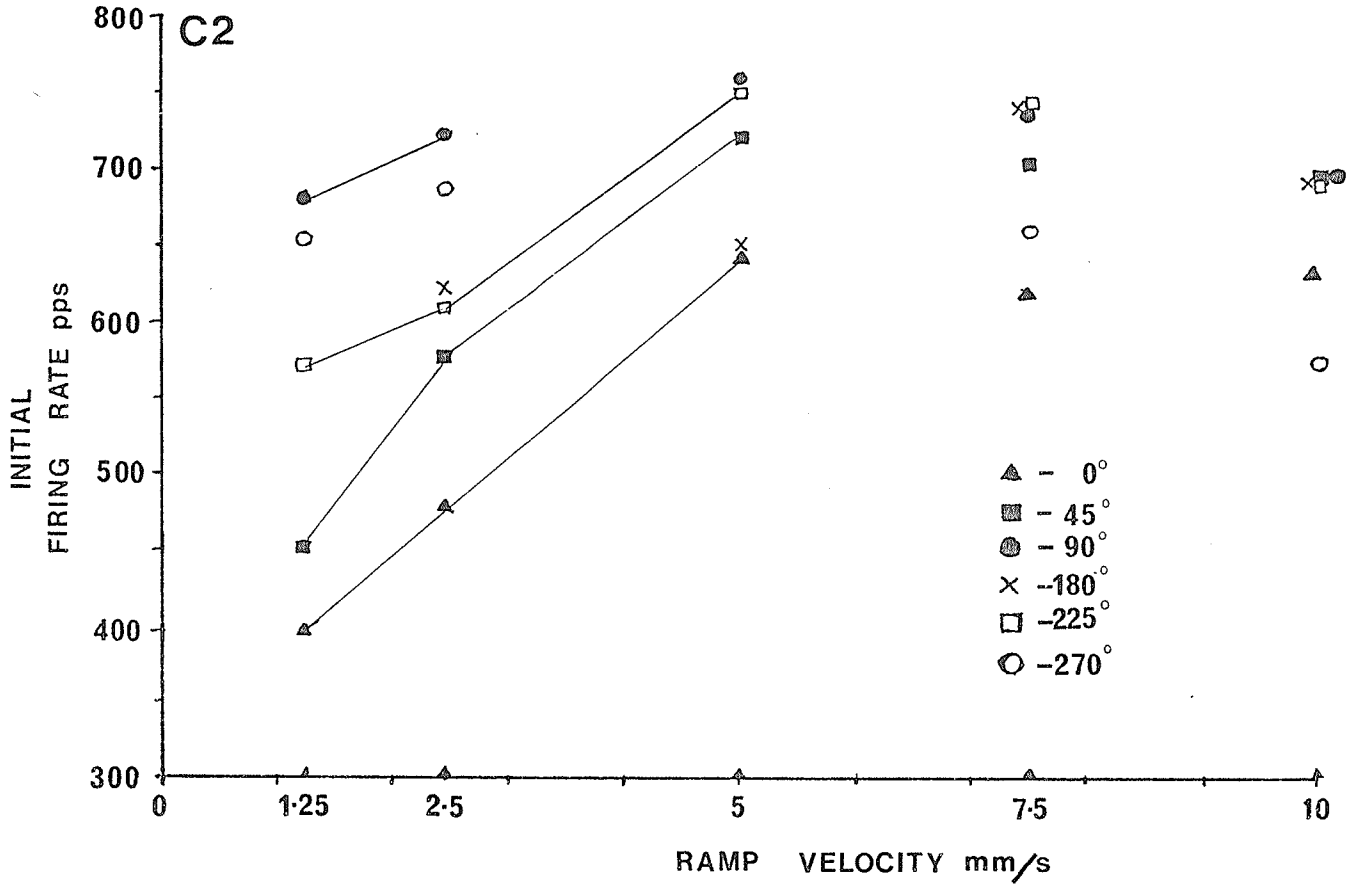

C2
800
700
600
500
400
300
INITIAL
FIRING RATE pps
0
1·25
2·5
5
7·5
10
RAMP VELOCITY mm/s
– 0°
– 45°
– 90°
–180°
–225°
–270°

Fig. 13. Average initial firing rate of a trochanteral hair sensilla (pps) versus ramp velocity plotted for various stimulus angles, all displacement values for each velocity being pooled. Each point represents the average of from 20 to 70 individual responses.

It can be seen that there appears to be a linear increase of firing rate with increasing velocity until at about 5 mm/sec the response saturates. Some directional sensitivity is evident with receptors at 90° being more sensitive than those at 0° and 45°.

per stimulus, over a range of stimulus parameters in the manner described in the methods section. Families of curves, such as those illustrated in Fig. 14, were obtained from averaging the responses to 10 sequential, identical stimuli for each series of displacements at a given ramp velocity (= one set of curves). It is evident from inspection that there exists a distinct relationship between displacement and impulse number.

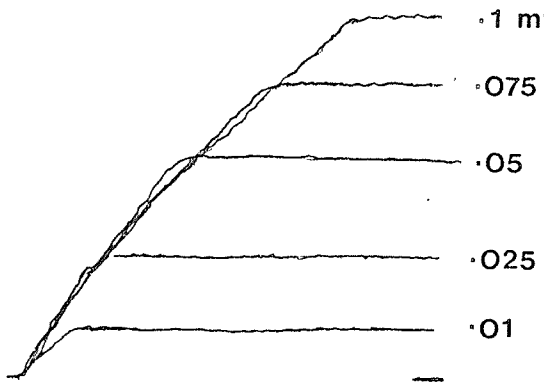
Determination of Stimulus-Response Relationship of Receptor

To permit the determination of the stimulus-response relationship, if any, in the face of the observed spread of response values (Fig. 15A), all results were normalized by setting the response to the maximum stimulus (displacement) of a given velocity series (R^*_{max}) at 100, and expressing other responses, R^*, as a percentage of this R^*_{max}, $S_{t\ max}$ is the maximum stimulus.

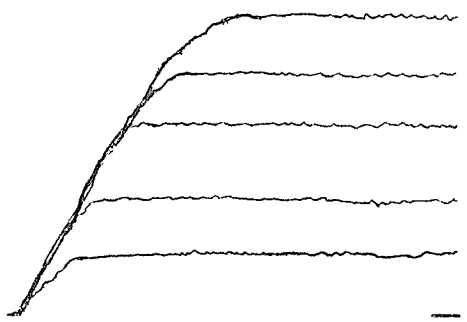
$$R = \frac{\text{Response } (R^*)}{\text{Response to } S_{t\ max} (R^*_{max})}$$

The effect of this operation can be appreciated from Fig. 15B in which a sample set of responses taken at random from a number of different preparations stimulated at various velocities, have been plotted in the raw and the normalized form. It is evident that the normalization procedure permits direct comparison of the stimulus-response relationship, while suppressing the effects of variation in the magnitude of the responses resulting from different animals, stimulus velocity and direction. Since it is not within the scope of this study

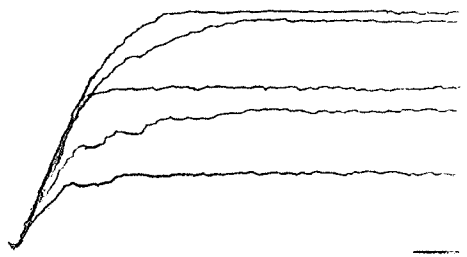
C2



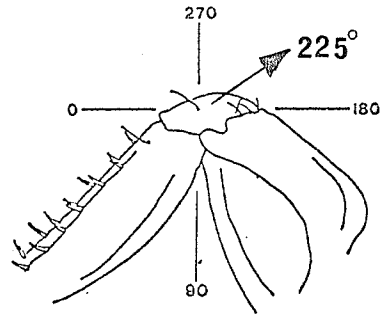
1·25 mm/sec



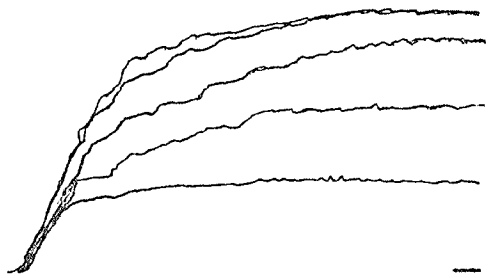
2·5



5



7·5



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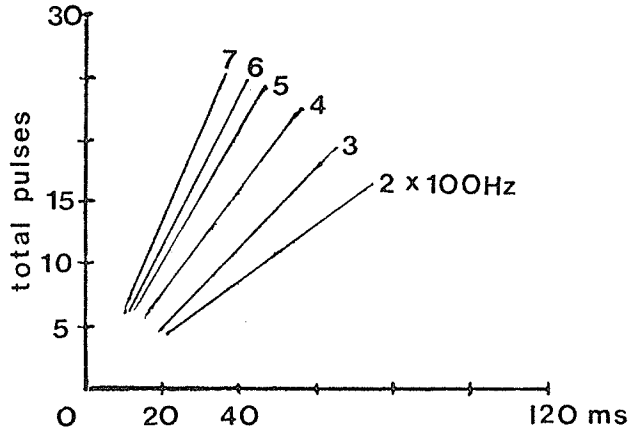


Fig. 14. Averaged cumulative counts for responses of the trochanteral hair sensilla (C2) to ramp mechanical stimuli of varying amplitude and velocity. Stimulus angle 225°.

Each family of curves represents the complete responses to varying displacement at a constant velocity, and each curve is the average of 10 responses.

In each set of curves, the order of displacement remains the same as that given in the top left hand set (1.25 mm/sec).

The slope of the six lines in the right band bottom corner is proportional to impulse frequency.

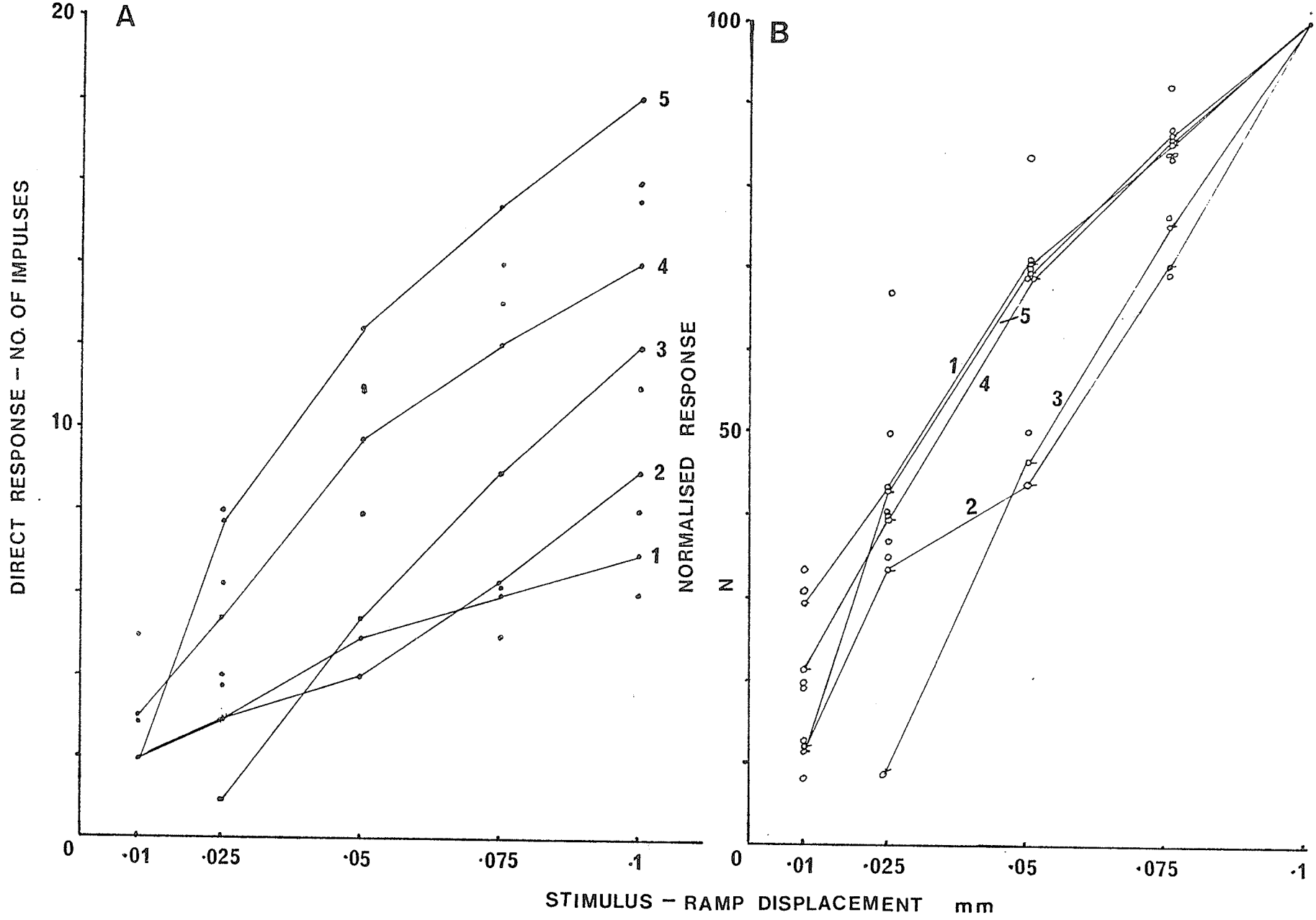
It can be seen that with increasing stimulus velocity, the receptor response shows saturation, as evidenced by the compression of the curves.

Fig. 15. Diagram illustrating the effect of the normalizing procedure on the raw data.

The responses have been chosen at random from the experimental data to illustrate the effects of the procedure and the range of the numbers of impulses per response (R^*) generated by the receptors.

The same five receptors have been connected by lines in the two plots.

It can be seen that normalizing permits comparison of the basic response function, in otherwise disparate results.


A
B
20
10
0
100
50
0
·01
·025
·05
·075
·1
1
2
3
4
5
DIRECT RESPONSE – NO. OF IMPULSES
NORMALISED RESPONSE
N
STIMULUS – RAMP DISPLACEMENT mm

to determine in great detail the variations of individual receptor responses vis a vis the generalized responses of a population of receptors, the information lost as a result of normalization is not important to this study.

On plotting the normalized receptor response data on linear co-ordinates, it became evident that the stimulus-response relationship appeared to fit a power function of the type described by Werner and Mountcastle (1965) for the mammalian 'Iggo's' corpuscle. A plot of the data on log-log co-ordinates revealed a straight line relationship indicating that indeed this receptor obeyed a stimulus-response relationship from the form

$$R^* = kS_t^n \quad \ldots\ldots\ldots 2$$

where R^* is the response expressed as the number of impulses; k is a constant of proportionality, and S_t is the stimulus intensity measured as displacement of the tactile hair.

The exponent n is determined from the slope of the fitted straight line to the log-log plot.

However, the normalization procedure modifies the power function above by cancelling out the constants k of the power functions of the individual receptors.

Let S = stimulus (S_t) as a percent of maximum stimulus (S_t max)

$$S = \frac{S_t}{S_t max} \times \frac{100}{1}$$

$$R = \frac{R^*}{R^* max} \times \frac{100}{1}$$

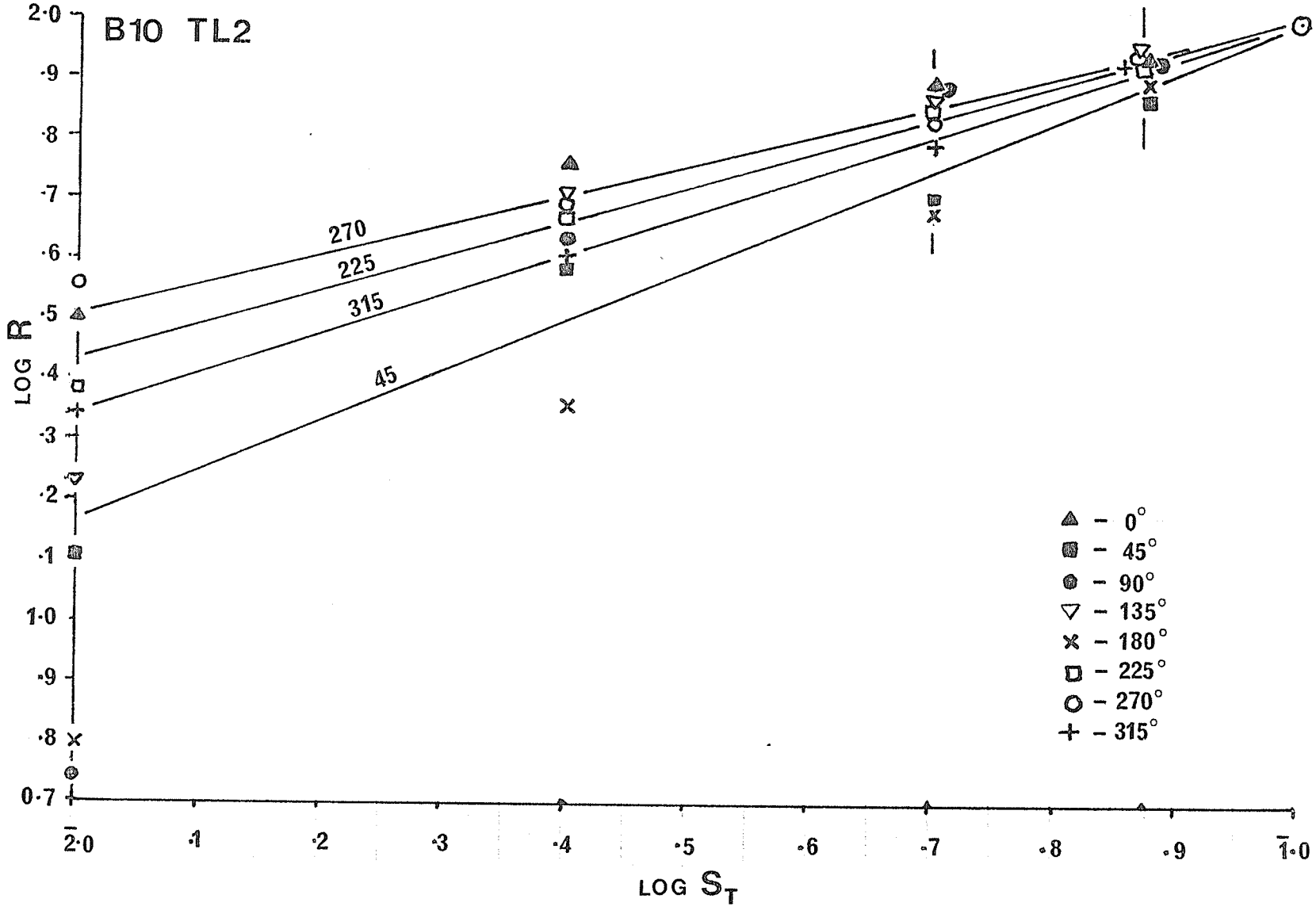

B10 TL2
2·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
1·0
·9
·8
0·7
LOG R
270
225
315
45
▲ – 0°
■ – 45°
● – 90°
▽ – 135°
× – 180°
□ – 225°
○ – 270°
+ – 315°
2·0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
LOG S_T

Fig. 16. Log-log plot of normalized responses of one trochanteral hair sensilla (left second thoracic leg bid), to ramp stimuli over the range 0.01 to 0.1 mm displacement; the responses plotted for each stimulus angle. Each data point contains the pooled responses to stimulus velocities and represents the average of individual responses.

It can be seen there is some variation of sensitivity with direction (as estimated by the slope of the curve), but this does not seem to follow a consistent pattern. It can also be seen that there is a straight line relationship between log S and log R for most of the responses, as shown by the fitted regression lines.

(45°:r^2 = 0.968, N = 15; 135° (not drawn in):r^2 = .863, N = 20; 225°:r^2 = 0.956, N = 20; 270°:r^2 = 0.979, N = 20; 315°: r^2 = 0.974, N = 15)

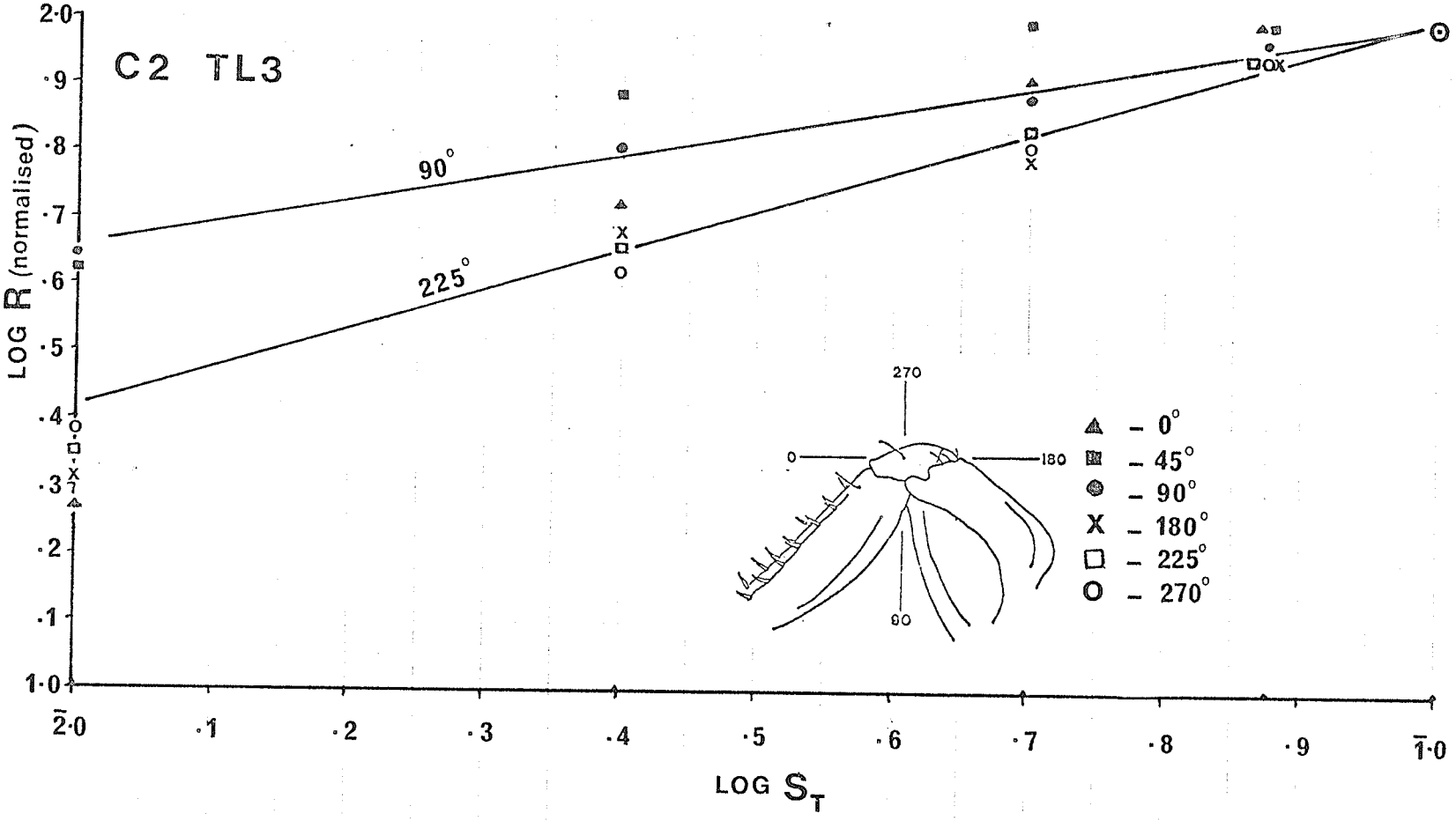

C2 TL3
LOG R (normalised)
2·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
1·0
90°
225°
270
0
180
90
▲ – 0°
■ – 45°
● – 90°
X – 180°
□ – 225°
O – 270°
2̄·0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1̄·0
LOG S_T

Fig. 17. Log-log plot of normalized responses of one trochanteral hair sensilla (left third thoracic leg), to ramp stimuli (conditions are the same as for Fig. 16).

As for the sensilla responses plotted in Fig. 16, this receptor also shows a linear relationship and approximately the same order of sensitivity, n.

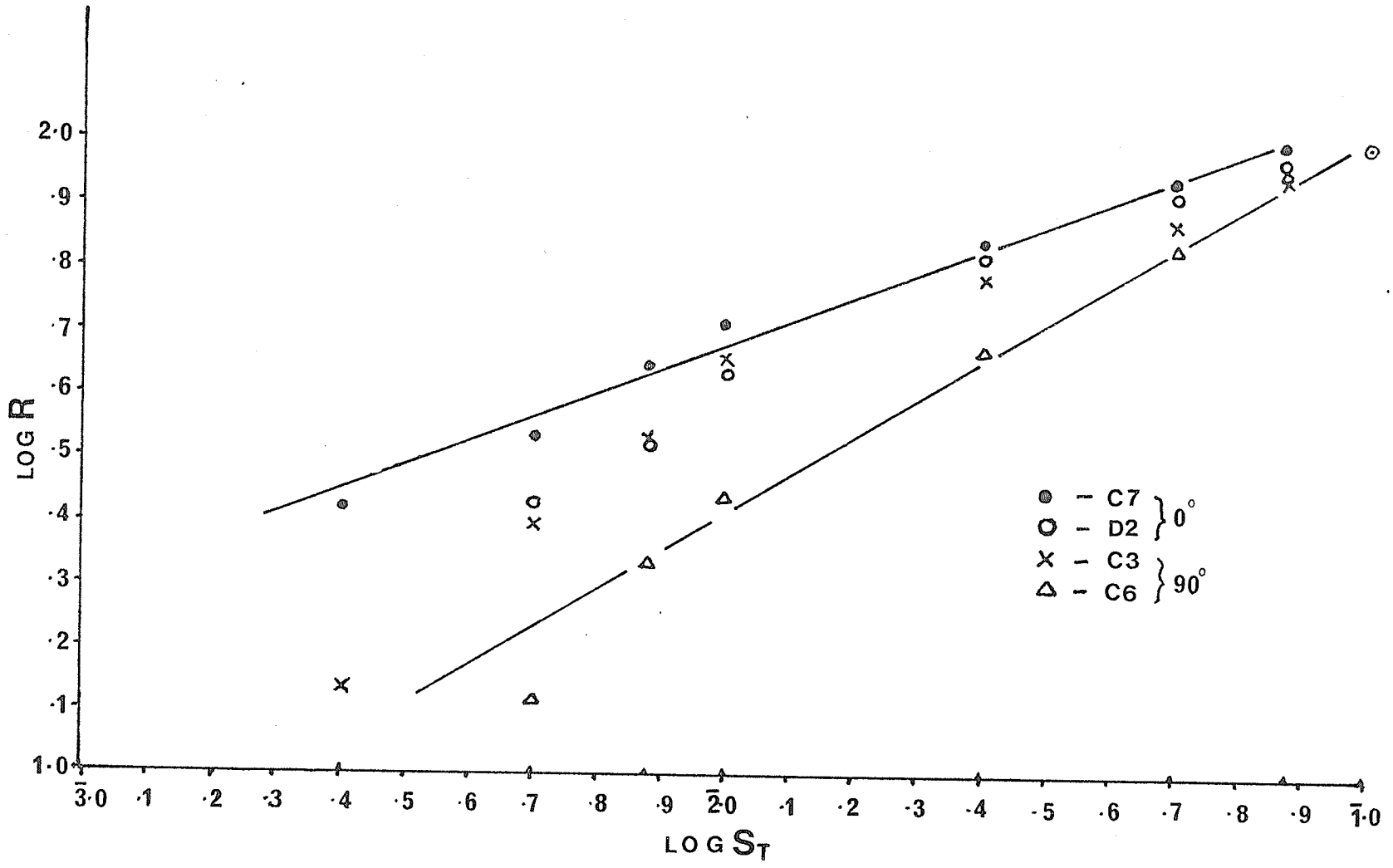
Regression lines from the regression analysis have been fitted to the responses obtained at two of the stimulus angles. $225°: r^2 = 0.927$, $90°: r^2 = 0.902$

Fig. 18. Log-log plot of normalized response for the trochanteral hair sensillae of four different animals to a wide stimulus intensity range (0.0025 mm to 0.1 mm displacement). The values for different displacement velocities have been pooled so that each point represents from 20 to 60 individual responses.

It can be seen that with the exception of C7, the actual responses deviate from a straight line fit at low stimulus intensities. C7 exhibits a lower sensitivity than the other sensillae, showing saturation of the response at displacements above 0.075 mm, but appears to have a lower displacement threshold.

Both of the regression lines fitted have been calculated from the exponential regression analysis.

(C7:r^2 = 0.891, N = 42; C6:r^2 = 0.939, N = 42)


LOG R
2·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
1·0
3·0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 2·0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0
LOG S_T
● – C7
○ – D2
} 0°
× – C3
△ – C6
} 90°

where R^* is the measured response, and R max the response to $S_t max$.

From equation 2

$$R = \frac{kS_t^n}{R^*max} \times \frac{100}{1} \quad \text{where } R^*max = k(S_t max)^n$$

$$= \frac{kS_t^n}{k(S_t max)^n} \times \frac{100}{1}$$

$$= \left(\frac{S_t}{S_t max} \times \frac{100}{1} \right)^n \cdot \frac{100}{100^n}$$

$$R = 100^{1-n} S^n \quad \ldots\ldots\ldots\ldots 3$$

In the pooled data, the exponent n becomes a weighted average of the exponents of the power functions of the original responses.

To determine the statistical validity of the power function as a description of the stimulus-response relationship of the hair, an exponential regression analysis was performed on the normalized data.

The equation fitted was of the form

$$Y = a_1 e^{b_1 X}$$

which corresponds in the terminology used above to

$$R = 100^{1-n} . e^{(n \log_e S)}$$

where $a_1 = 100^{1-n}$; $b_1 = n$; $X = \log_e S$ and $Y = R$.

It was considered more suitable to fit the regression equation in the exponential rather than the linear form because

of the nature of the error terms. The error terms of the observations about the exponential regression line appear to be additive and approximately normally distributed.

To fit the log-log relationship, the error terms must be multiplicative so that after the log-log transformation they become additive. Also, the log transformation of normally distributed error terms will be highly skewed and not normally distributed.

The regression analysis program was run both on the responses of individual animals to different stimulation directions and on the pooled data of 82 sets of hair responses from 12 animals.

The values for the coefficient of determination r^2, a_1, b_1 and the second independent estimate of n obtained from a_1 for all the regressions performed are listed in Table 1.

In all cases, the proportions of the total error explained by the regression r^2 was above 0.85 with a sample size of 15 or more, and the plotted fits looked excellent (Appendix 1).

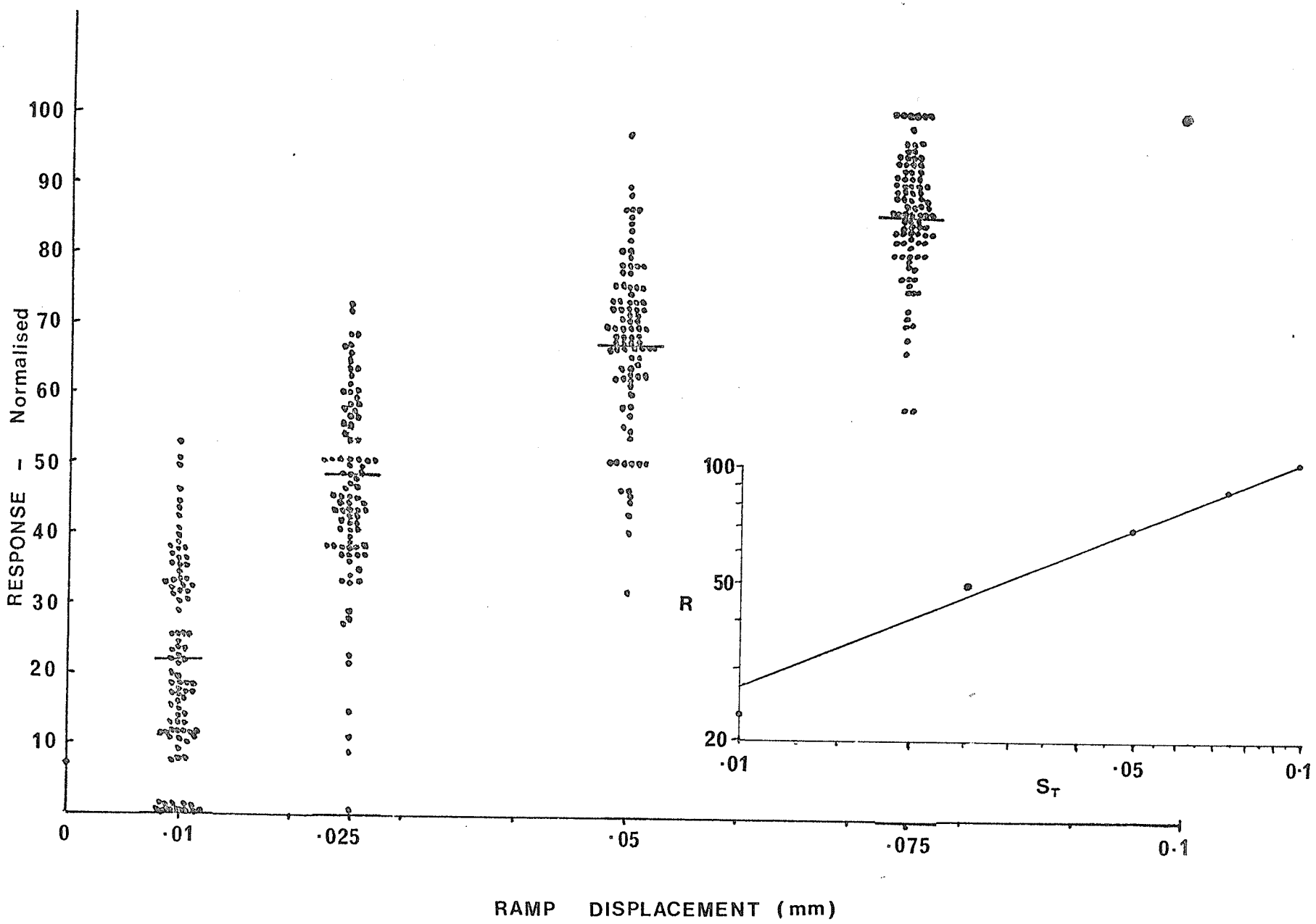
The use of the F test in this non-linear regression may not be strictly admissable; however, if the F values are calculated, in all cases r^2 is significantly different from zero, that is the regressions are significant at least at the 0.5% ($P = 0.005$) level.

It is interesting to note that the two independent estimates of the exponent n, obtained from a_1 and b_1 are in close agreement. This increases the confidence one may place

Fig. 19. Scatter diagram of the normalized response of 12 sensillae. All responses have been pooled without regard for animal, hair position, stimulus orientation, or stimulus velocity. Each of the 410 data points is the average of 8 to 10 individual responses. Horizontal bars indicate arithmetic means.

The inset graph is a plot of the arithmetic means of the pooled data plotted against stimulus displacement S_t on logarithmic axes, to which a regression line calculated from the exponential regression analysis has been fitted ($r^2 = 0.889$ for $N = 410 - P > 0.001$).

It is evident that since the points lie on the straight line, the stimulus-response relationship can be described by a power function of the general form $R = kS_t^n$ where n is given by the slope of the fitted line and in this case is 0.625.


RESPONSE - Normalised
100
90
80
70
60
50
40
30
20
10
0
·01
·025
·05
·075
0·1
RAMP DISPLACEMENT (mm)
R
100
50
20
·01
·05
0·1
S_T

in the validity of the regression, i.e., that the stimulus-response relationship of the receptors studied is a power function.

TABLE 1

Analysis	r^2	a_1	b_1	Estimate of n from a_1	No. obs.
B 10 45°	0.968	2.192	0.824	0.830	15
B 10 135°	0.863	7.071	0.585	0.575	20
B 10 225°	0.956	7.103	0.577	0.574	20
B 10 270°	0.979	10.48	0.487	0.490	20
B 10 315°	0.974	4.697	0.667	0.664	15
C 2 90°	0.902	19.71	0.355	0.353	25
C 2 225°	0.929	6.553	0.597	0.592	30
C 6	0.939	6.685	0.594	0.587	42
C 7	0.891	19.69	0.382	0.353	42
Pooled	0.889	5.682	0.625	0.623	410

If we define sensitivity η as a proportional change in response R^* for a small proportionate change in stimulus S_t, the exponent n determined from the regression analysis is equal to the sensitivity.

That is:

$$\eta = \frac{\frac{dS_t}{S_t}}{\frac{dR^*}{R^*}}$$

$$= \frac{S_t}{R^*} \cdot \frac{dR^*}{dS_t}$$

$$= \frac{S_t}{R^*} \cdot (knS_t^{n-1})$$

$$= n$$

Note also that η also equals $\frac{\frac{dS}{S}}{\frac{dR}{R}} = n$.

For a given animal, the response to an increase in absolute stimulus magnitude varies with the magnitude of the stimulus. However, the percentage change in response for a percentage change in stimulus is constant as demonstrated above. Also, only with the index of percentage change, n, can we compare receptors. Thus, it is not practicable to define sensitivity in absolute terms of impulses/mm.

From Table 1 sensitivites for the sensillae investigated range from 0.355 to 0.824. The n for the pooled data, which represents the averaged sensitivity for the population, is 0.625.

Some alterations in sensitivity with different stimulus directions are evident (Figs. 6, 17, 18), but again, as for the effects on firing frequency, these changes do not appear to follow any consistent pattern.

While the majority of the measurements were carried out over the displacement range 0.01 to 0.1 mm, 4 preparations were tested at smaller displacements after modifications

to the ramp generator. The results for these, as well as the regression lines fitted from the regressions performed on two of the sets of results, are given in Fig. 18. It can be seen that the power function is followed down to displacements as small as 2.5 microns.

Receptor Potentials

Since this receptor is of the rapidly adapting velocity sensitive type (Wolbarsht 1960) which is characterized by the fact that it produced an output only when the stimulus is changing, it was of interest to compare the receptor potential with those obtained from a variety of insect hair receptors by Wolbarsht (1960).

A rigorous qualitative evaluation of the relation between stimulus, receptor potential and spike generation was not attempted in this study.

Fig. 5B shows the general pattern of the receptor response. It can be seen that the receptor potential averaged 2.0 millivolts, a value in agreement with the values found by Wolbarsht. However, the action potentials are very small ($\simeq$ 50 μv) in comparison to those recorded by Wolbarsht (2 μv) which suggests that the spike initiation region is separated from the receptor region of the sensory neurone, either by a great distance or a region of high resistance. Unfortunately, it was not feasible to measure the resistance of the hair shaft, or to determine the anatomical details of the receptor in order to support either idea.

From Fig. 20, it can be seen that the amplitude of the receptor potential is a function of the velocity of the ramp stimulus, a relationship that agrees with the observed fact that firing frequency is a function of ramp velocity (Fig. 13). Wolbarscht's observations that the receptor firing frequency is a linear function of the receptor potential explains the observed relationship well.

The duration of the receptor potentials shown in both Fig. 5B and Fig. 20 are of longer duration than the rising phase of the ramp stimulus. It is also of interest to note that the 'off' response is of the same polarity as that produced in response to the ramp. This would argue for two possible mechanisms underlying the adaptation exhibited by this receptor, either the ionic permeability of the generator region shows rapid recovery after distortion, and that it responds to any distortion regardless of direction, or there is a mechanical change underlying adaptation as suggested by Crowe (1967).

Tonic Displacement Receptors

Several animals of the 30 or so investigated exhibited evidence of tonic responses to extreme sustained displacement of the hair sensilla. Unfortunately, a systematic investigation of all the preparations was not carried out so that it is impossible to state what proportions of the receptors possessed this response. The effective stimulus appeared to be an extreme displacement of the hair so that it lay almost

parallel to the surface of the cuticle. Fig. 21 illustrates such a response, the receptor can be seen to fire in pulse pairs at an initial rate of 6 bursts per second, declining to 4 per second after 10 seconds.

The amplitude of these action potentials appears to be the same as those produced by the phasic receptor, although there is no reason to presume that they are from the same receptor cell.

No quantitative study of the nature of these responses was undertaken. Hair receptors having multiple tactile responses do not seem to have been described from insects (Dethier 1963) but have been described from scorpions (Sanjeeva-Reddy 1971). Most often the hairs respond to two different sensory modalities, e.g., combined chemosensory and tactile receptors (Larson 1962).

Thermal Responses

Bringing a fine (0.1 mm diameter) heated needle (100°C approximately) into the near vicinity of the tactile hair base (0.5 - 1 mm) caused an abrupt onset of activity (Fig. 22). However, it is not possible to assign the source of these impulses specifically to the hair receptor neurones in the absence of detailed histological studies.

Freely Walking Preparation

Unfortunately, despite many attempts to eliminate interference from the motor axons which run as part of Nerve 5 through the trochanter by various electrode placements in conjunction with a differential amplifier, it was not possible

E5

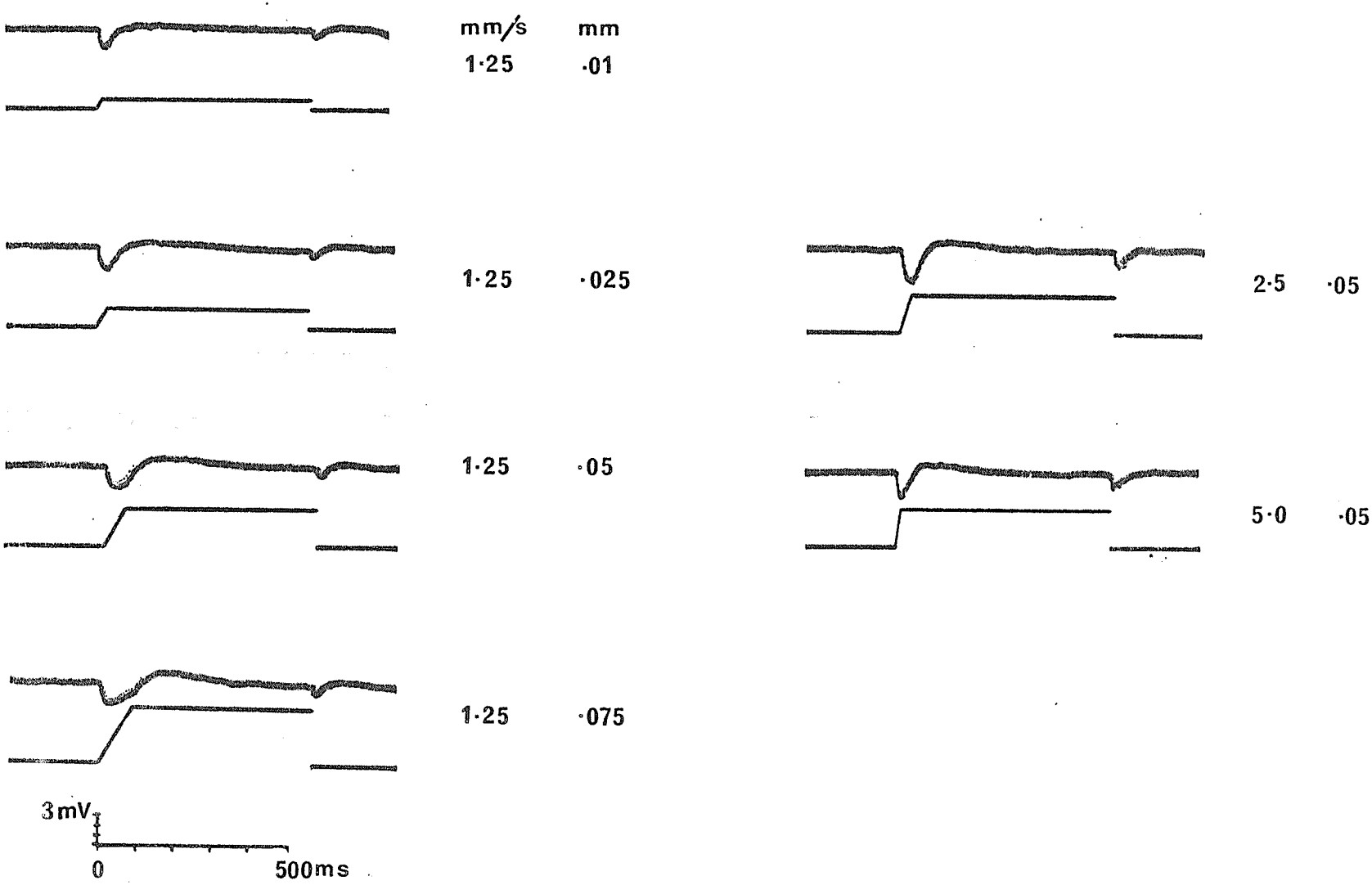


Fig. 20. Continuous film records of receptor potentials developed in response to ramp mechanical stimuli of different velocities and displacements. The phasic nature of the response is evident, as is the increase in the receptor potential duration with increasing stimulus displacement. However, the receptor amplitude appears to plateau with increasing displacement at 1.25 mm per second, but shows an increase in amplitude with increasing velocity.

A

B

0 1 Sec

C

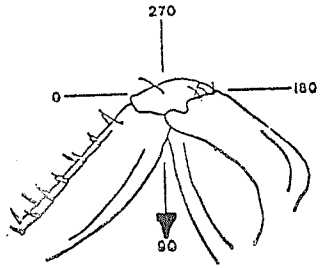


Fig. 21. Tonic responses of a secondary receptor to prolonged extreme mechanical displacement of a trochanteral hair sensilla, left third thoracic leg, the sensilla being bent in the direction of arrow (inset) until it was almost parallel with the surface of the cuticle. Note that the receptor fires in bursts of two or three spikes.

A. Response following displacement of the hair - the rapid firing at the start is due to the movement of the hair, and is probably from the phasic unit.

B. Discharge rate after 10 seconds of maintained deflexion.

C. Response of the phasic component to touching with a fine paint brush for comparison.

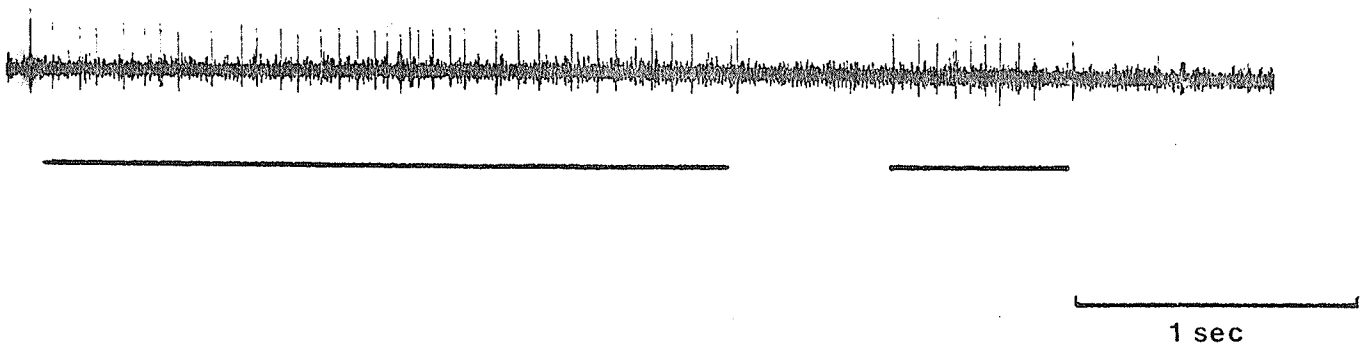

1 sec

Fig. 22. Responses of the same preparation as in Fig. 21. To radiant heating from a fine (0.1 mm diameter) black electrically heated needle having a temperature of approximately 100°C and held at about 0.5 mm from the hair base and hair. Heavy lines under the trace indicate the duration of the stimulus.

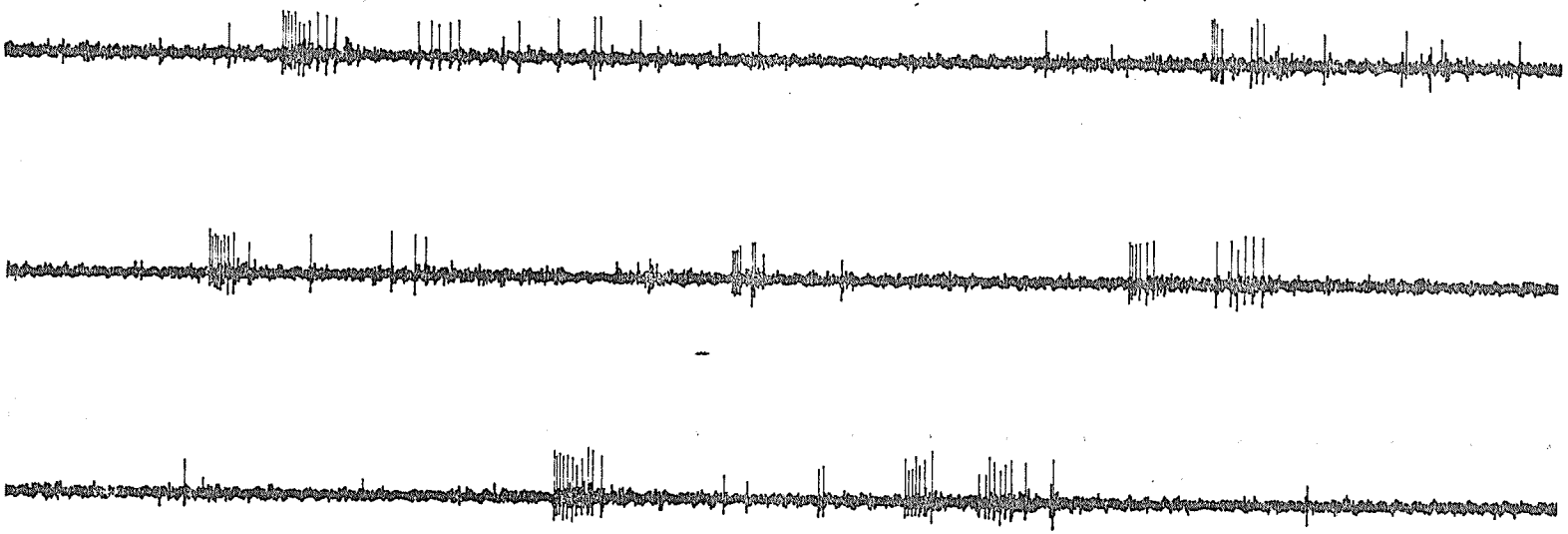
to record responses from the receptor when the animal was in active motion. Only when the limb was passively protracted as the animal moved forward, were any distinct receptor responses recorded.

Only when the animal was in the low position (Plate 1) did scratching or jarring the substrate elicit any responses (Fig. 24).

Only one spontaneous response that could be unequivocably assigned to the receptor was recorded (Fig. 24). This occurred when the leg appeared to brush against some object on the substrate while the leg was being protracted.

While it was possible to continuously record the leg nervous activity on tape for protracted periods, it was never possible to be fully certain of the source of discharges which resembled those of the receptor, unless one was able to directly observe contact being made.

A



B

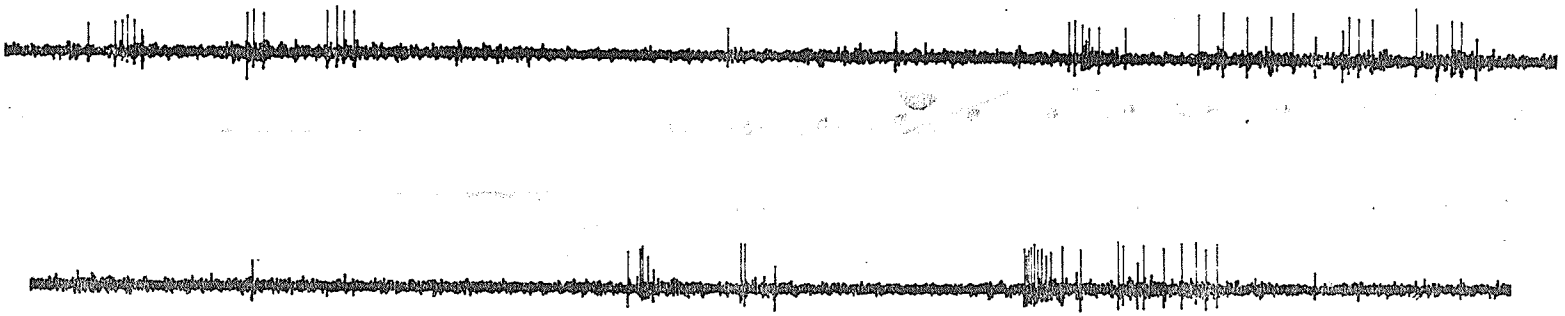


Fig. 23. Continuous film recording from unrestrained animal, the recording electrode in the trochanter of the second left thoracic leg in close proximity (c 200 microns) to the base of the trochanteral tactile hair. Pulse amplitude approximately 300 microvolts.

A. Response to gently touching the sensilla with a fine (00) camel's hair brush, the animal resting on the side of the constraining beaker. No overt behavioural response was elicited by this maneouver.

B. Response of the same animal to touching the sensilla with a brush as before, the animal resting on the bottom of the beaker.

Time marks 10 ms apart.

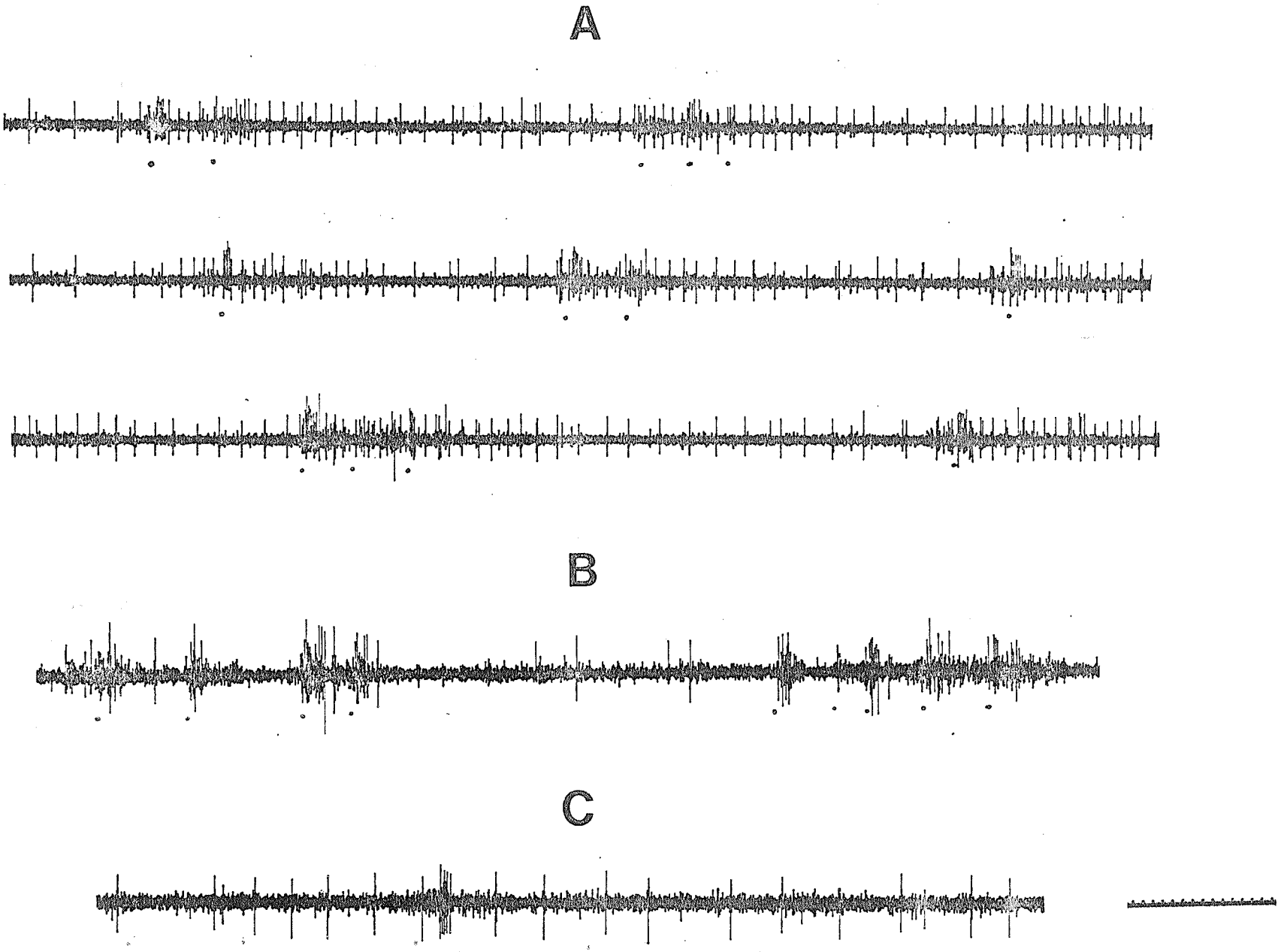

A
B
C

Fig. 24. Continuous film recording from unrestrained animal, recording conditions as described in Fig. 23. Dots under the trace indicate the approximate onset of the stimulus.

A. Response of the receptor to gently tapping the bottom of the constraining beaker on the bench, the animal being in the low posture (see Plate 1). The increase in the discharge rate of the tonic unit, presumably a postural motor neurone, cannot be ascribed to the receptor stimulation, but to the overall stimulation caused by the jarring.

B. Response to gently scraping the paper on which the animal was resting (low position), with the handle of the paint brush. There were no observable behavioural responses to this.

C. Spontaneous response from the receptor, the leg being in protraction while the animal was walking on the paper disc in the beaker. This is one of the few spontaneous responses observed.

Timing marks 10 ms apart.

DISCUSSION

From the data presented, it is difficult to come to any definite conclusion as to the role of these hair sensillae. The prominent position of the receptors on the ventral surface of the animals' limbs would make them ideal candidates for contact receptors, whether mechanical or chemosensory.

That they are not chemoreceptors appears to be the case from the complete lack of success in attempts to stimulate them chemically.

Stimulation of the receptor in resting unanaesthetized animals produced no overt responses, either in reflex leg nerve activity, or in behavioural responses. Sanjeeva Reddy (1970) has suggested that this is probably due to the considerable convergence of tactile sensory input to the insects CNS. As a result of this, the minimum effective stimulus would require the simultaneous stimulation of a number of receptors. This requirement is in contrast to the response to chemosensory hair stimulation in many insects in which stimulation of one labellar hair can release feeding behaviour (Dethier 1963).

As illustrated in Plate 1, the resting cockroach often assumes a low posture in which the tips of the long sensory hairs are in contact with the substrate, in a manner that would suggest that they function as vibration receptors. However, Schneider (1950) (as quoted in Dethier 1963), has demonstrated the existence of an exquisitely sensitive substrate vibration detecting mechanism in the form of the sub-

genual organs in each leg. The sub-genual organs are chordotonal organs which respond to the relative motions of the distal segments of the leg and the body, which acts as an inertial mass. In the cockroach, this organ can respond to displacement of 10^{-9} cm, at 1,500 cps. In the face of the existance of such an organ it is hard to postulate the need for another. In addition, Florentine (1967, 1968) has described what he claims to be abdominal vibration receptors in the cockroach.

Although it was not possible to fully characterize the frequency response of the hair receptors, it is probable that they are capable of responding synchronously to frequencies well in excess of 300 cps, since the receptor firing frequency in response to 5 mm/sec ramp stimuli ranged from 650 - 750 pps.

If this is the case, the hairs probably function simply as fast phasic tactile receptors when the animal is walking.

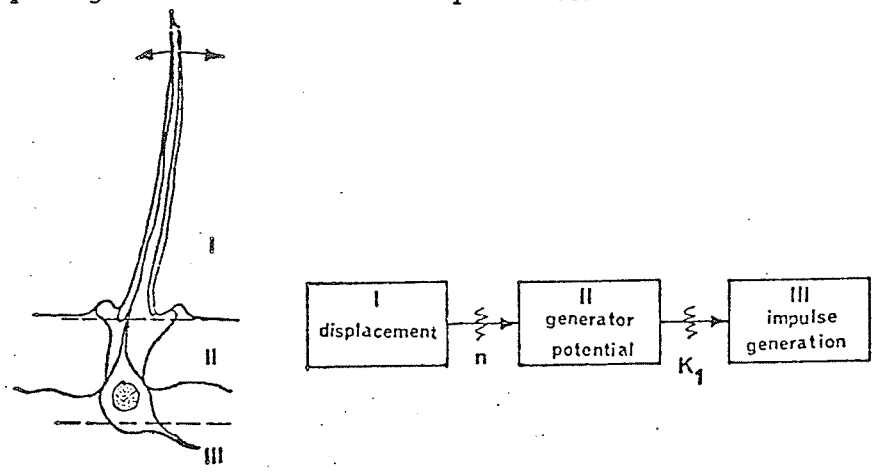
From the analysis of the responses to ramp stimuli, the receptor encodes both rate of hair displacement and degree of displacement, the latter in accordance with a power law relationship. However, since natural stimuli are seldom if ever ramp stimuli, this degree of coding accuracy may be of no physiological significance to the animal, and as the response is a function of both stimulus and displacement, there is also a high degree of ambiguity in the receptor response. Schwartzkopff (1964) has pointed out that it highly probable that the information from tactile receptors of importance to the insect is the time of contact and the anatomical position of the hair involved. Schwartzkopff contends that the intensity

of the stimulus is not first order importance.

This contrasts with the situation in mammals in which the touch corpuscle (Iggo's corpuscle) (Werner and Mountcastle 1965) encodes stimulus intensity, and there is little distortion of this coding in the thalamic relay neurones.

Due to the level of motor neurone and muscle activity which occurs during active movement of the animal, it has not been possible to establish the role of the hairs using free walking preparations.

The analysis procedure used in this study, using a ramp mechanical stimulus, would appear to permit the separation of two components of the transduction process in the hair sensillae. For this, the receptor may be considered in terms of a simple compartmental model with an assumption of a uniform mechanism underlying the production of the generator potential, for all sensillae in a population of animals. In other words, for an identical distortion of the receptor region, there is developed an identical generator potential. Given this assumption, it appears to be possible to ascribe variations in the observed responses to variations in the coupling between these compartments.



From the results, the value of n, the exponential term of the power function, would appear to reflect the degree of mechanical coupling between the stimulus and the generator region. This may be due to variations in shaft length, flexibility compliance of the socket, etc. That this may be the case is reflected in the variations in n with the direction of the stimulus.

It is difficult to account for the spread of the observed response values, R^*, for receptors given identical stimuli by the spread in n, the values for which are relatively constant for the receptors analyzed. It would appear that the spread in R^* reflects the spread in values of the constant K_1 in equation 1, and that K_1 is thus an index of the degree of coupling between the generator potential and the spike initiating region. Regrettably, it was not possible in this study to perform the extensive statistical analyses which would be required to separate out the effects of n and K_1 on the observed R to permit confirmation of this hypothesis.

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Appendix 1

Computor plots of the regression curve and the data points for each of the sets of receptors analyzed.

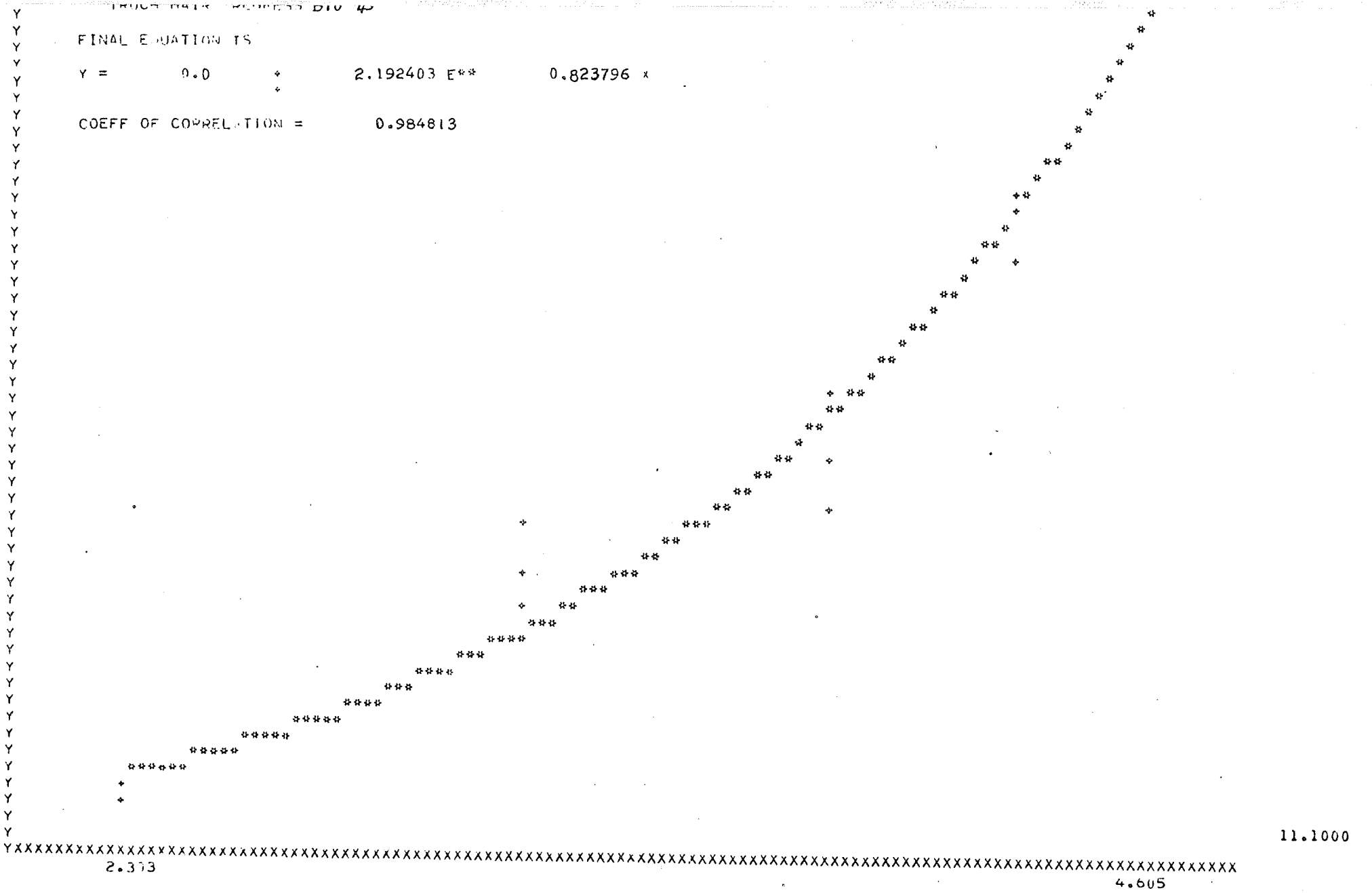
The X axis is calibrated in terms of $\log_e S_t$.

TRUCK RATE - BUSINESS BIO 45

FINAL EQUATION IS

Y = 0.0 + 2.192403 E** 0.823796 x

COEFF OF CORRELATION = 0.984813

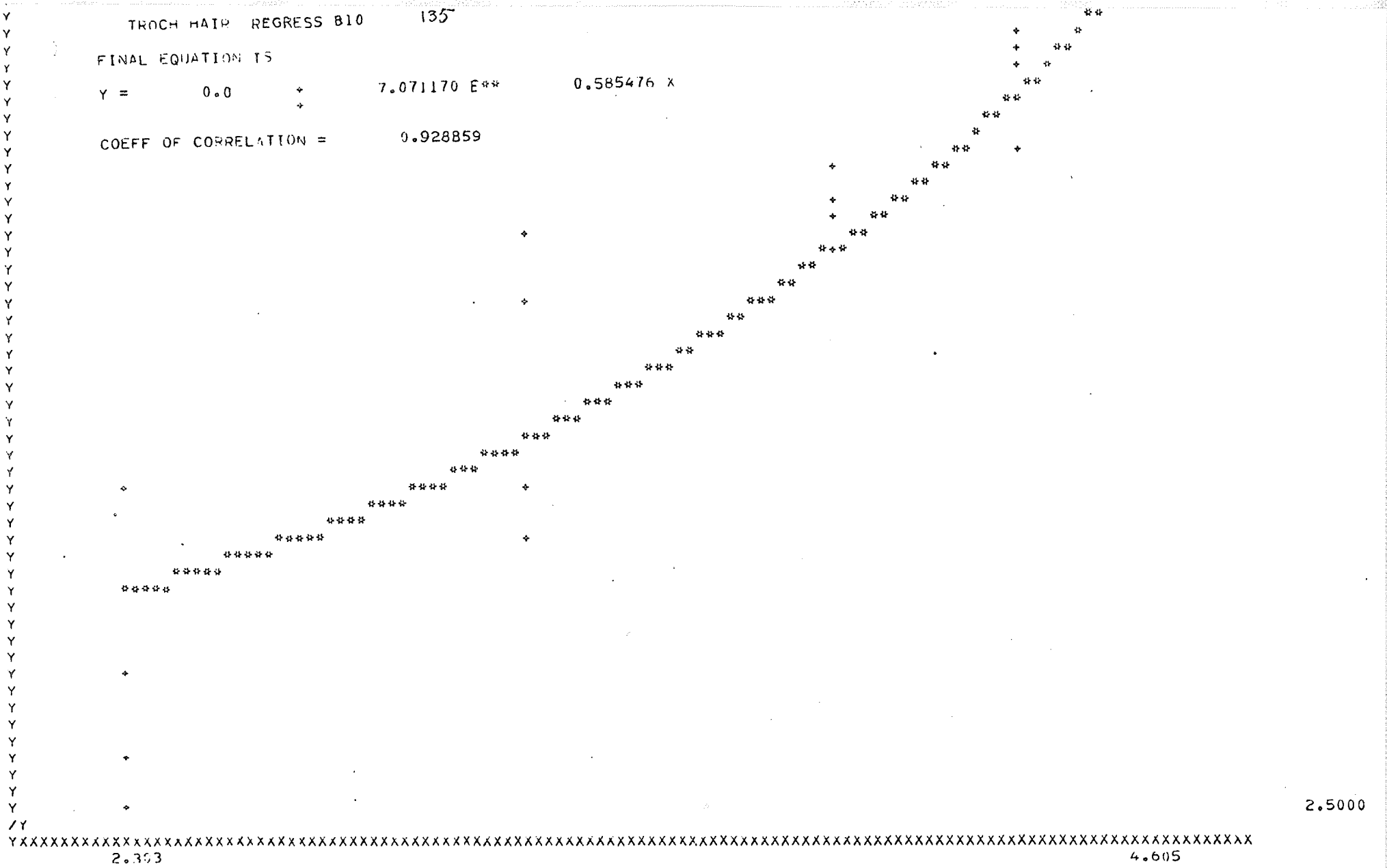


TROCH HAIR REGRESS B10

FINAL EQUATION IS

Y = 0.0 + 7.071170 E** 0.585476 X

COEFF OF CORRELATION = 0.928859



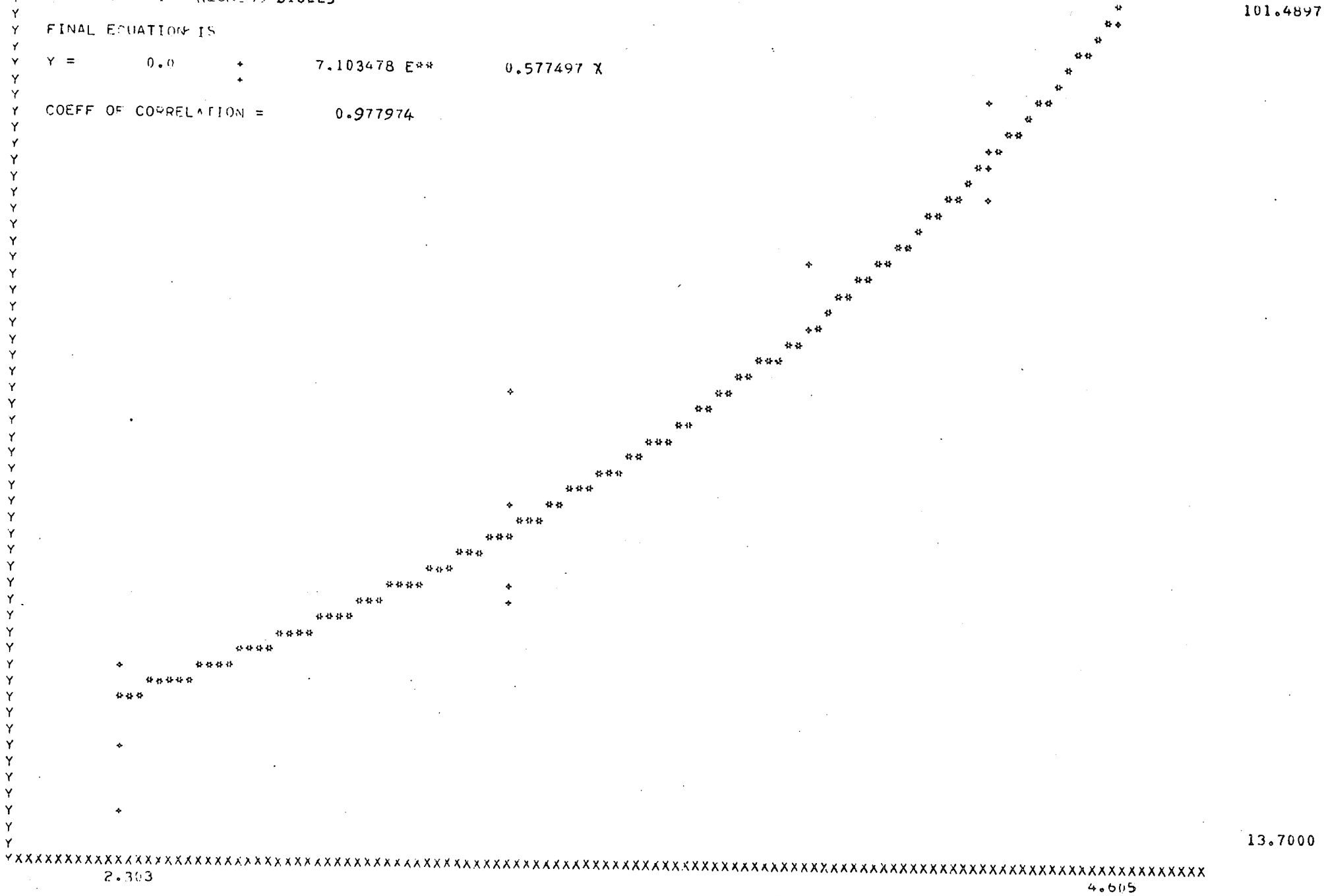
***** UNIVERSITY OF MANITOBA ***** EXPONENTIAL REGRESSION

TROCH HAIR REGRESS B10225

FINAL EQUATION IS

Y = 0.0 + 7.103478 E** 0.577497 X

COEFF OF CORRELATION = 0.977974



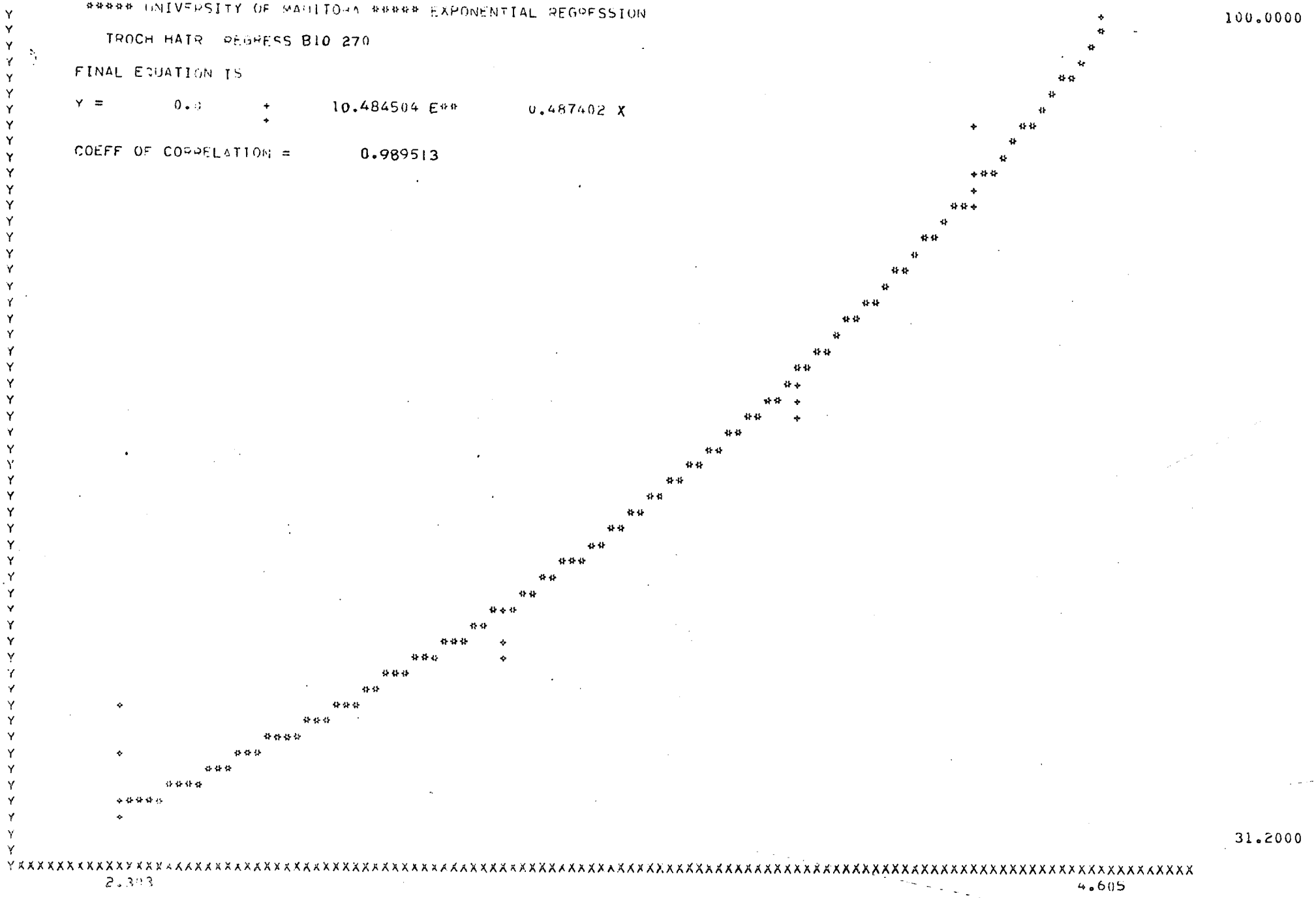
***** UNIVERSITY OF MANITOBA ***** EXPONENTIAL REGRESSION

TROCH HAIR REGRESS B10 270

FINAL EQUATION IS

Y = 0.0 + 10.484504 E** 0.487402 X

COEFF OF CORRELATION = 0.989513



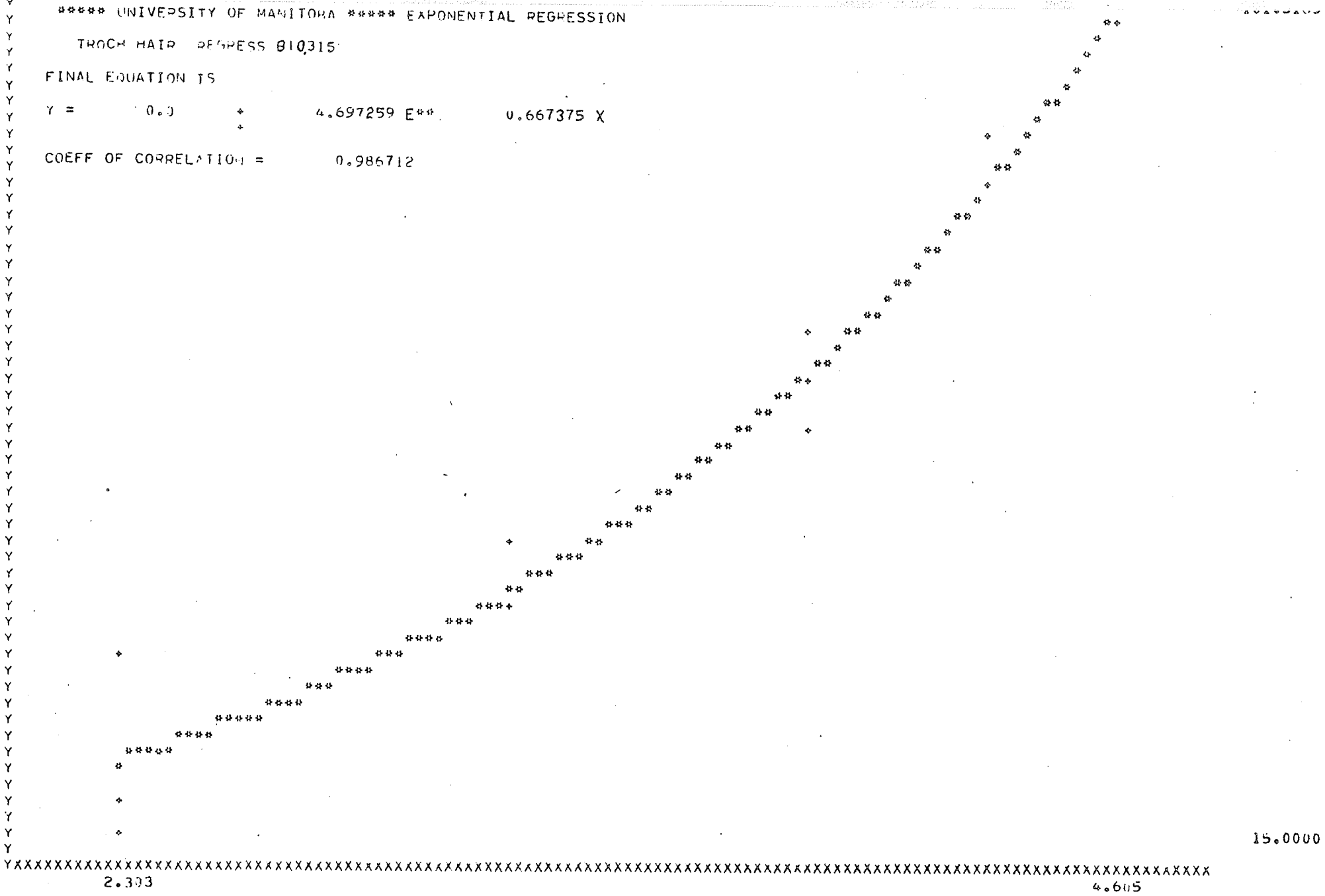
***** UNIVERSITY OF MANITOBA ***** EXPONENTIAL REGRESSION

TROCH HAIR REGRESS 810315

FINAL EQUATION IS

Y = 0.0 + 4.697259 E** 0.667375 X

COEFF OF CORRELATION = 0.986712



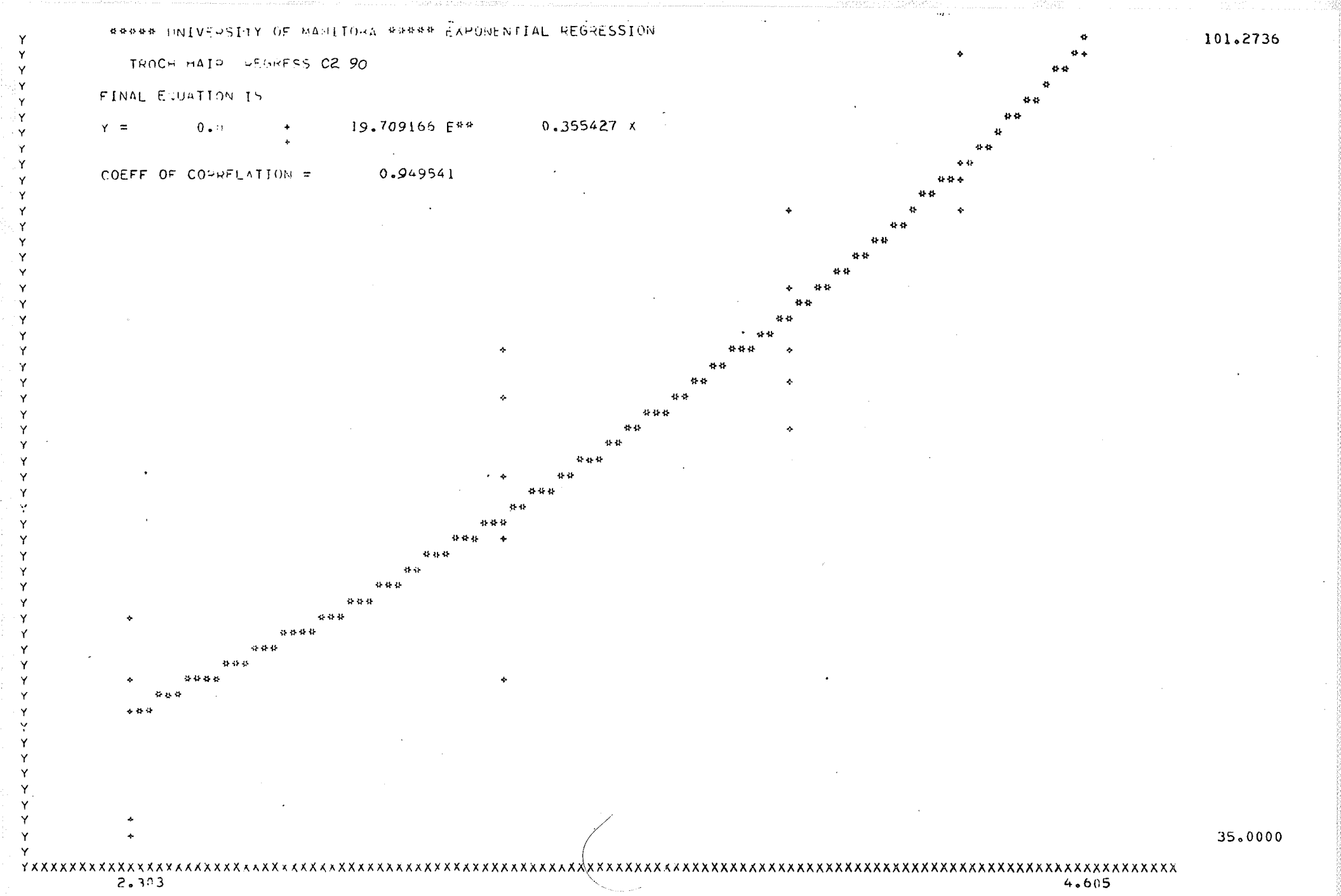
***** UNIVERSITY OF MANITOBA ***** EXPONENTIAL REGRESSION

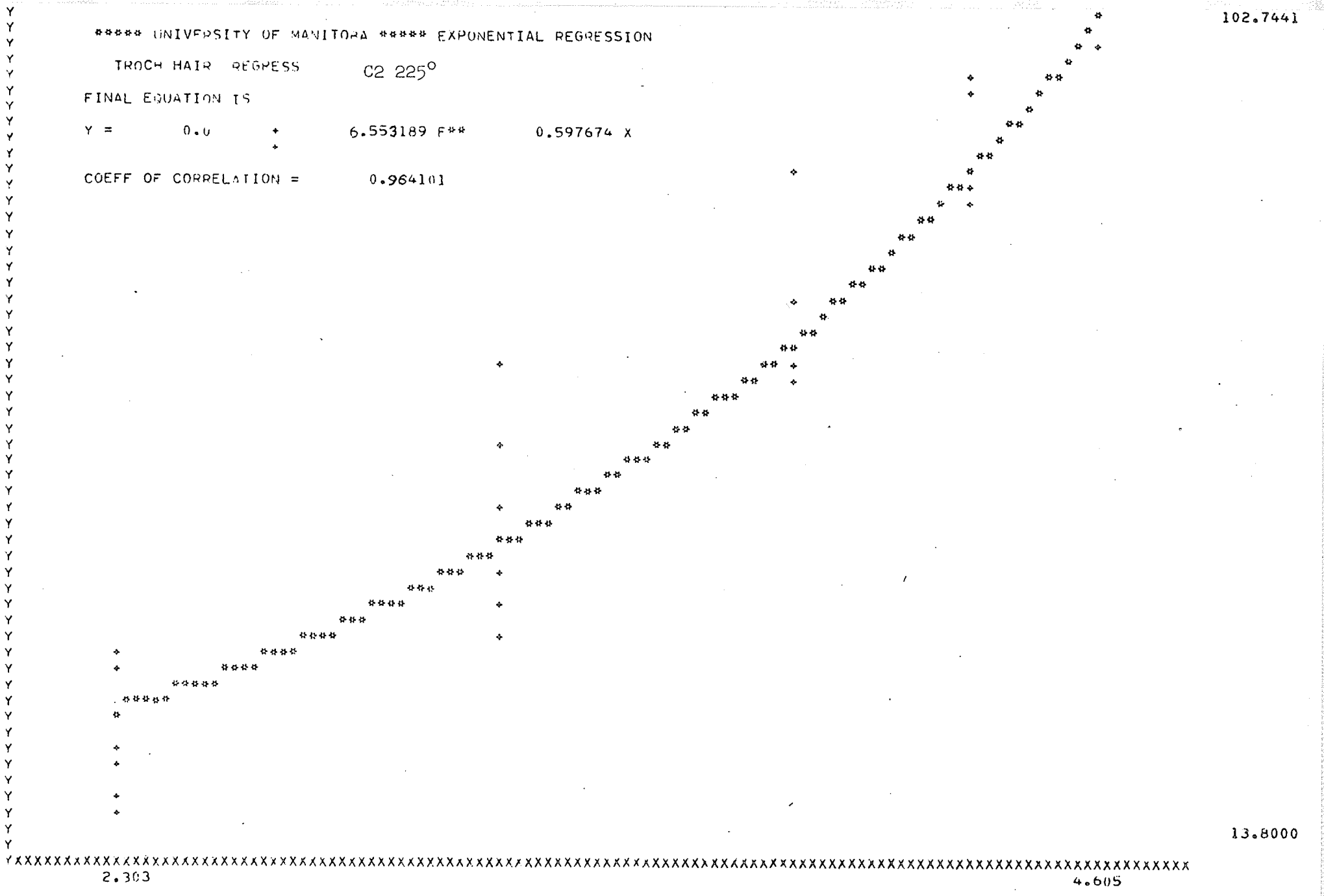
TROCH HAIR REGRESS C2 90

FINAL EQUATION IS

Y = 0.0 + 19.709166 E** 0.355427 X

COEFF OF CORRELATION = 0.949541

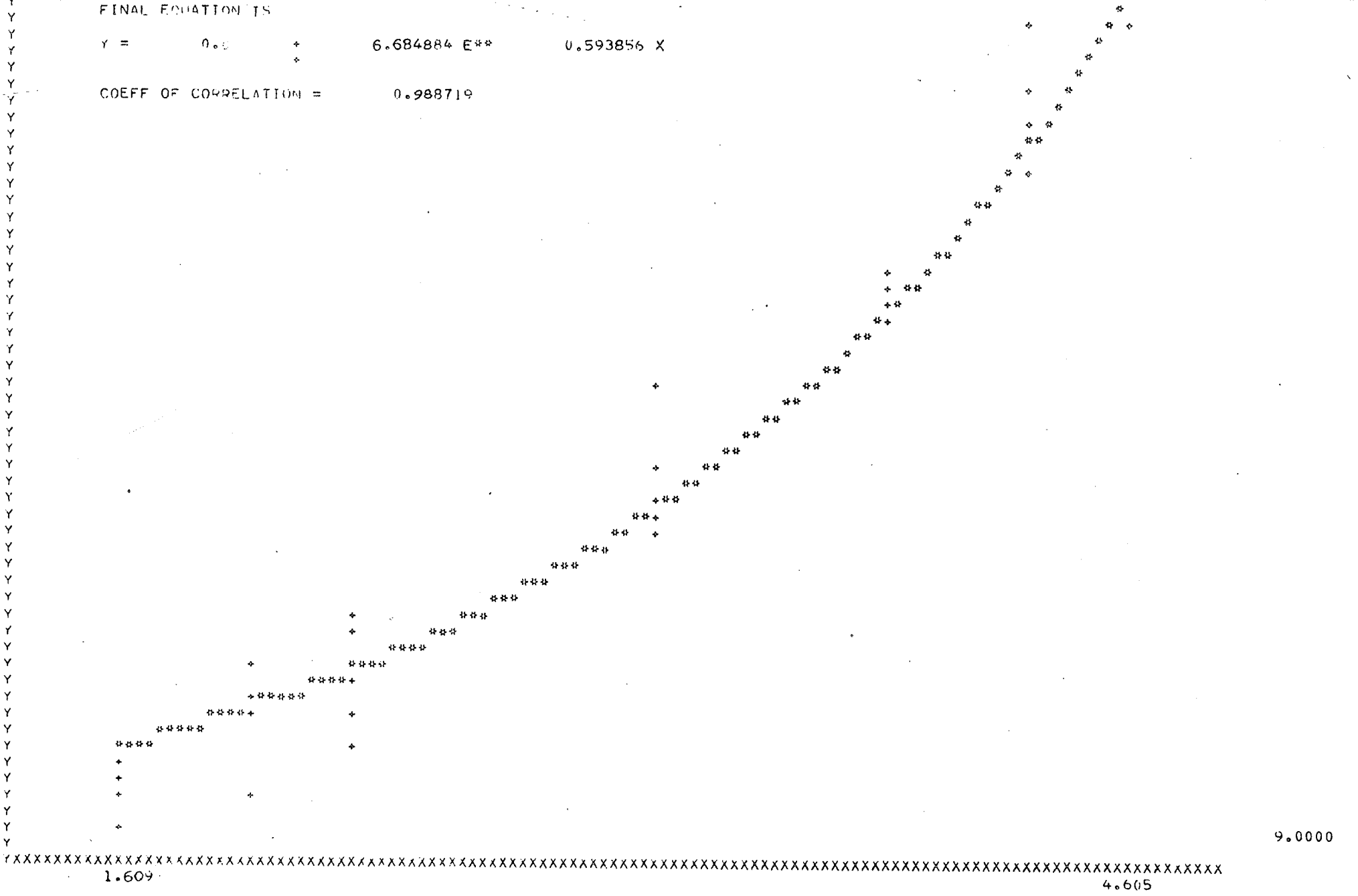


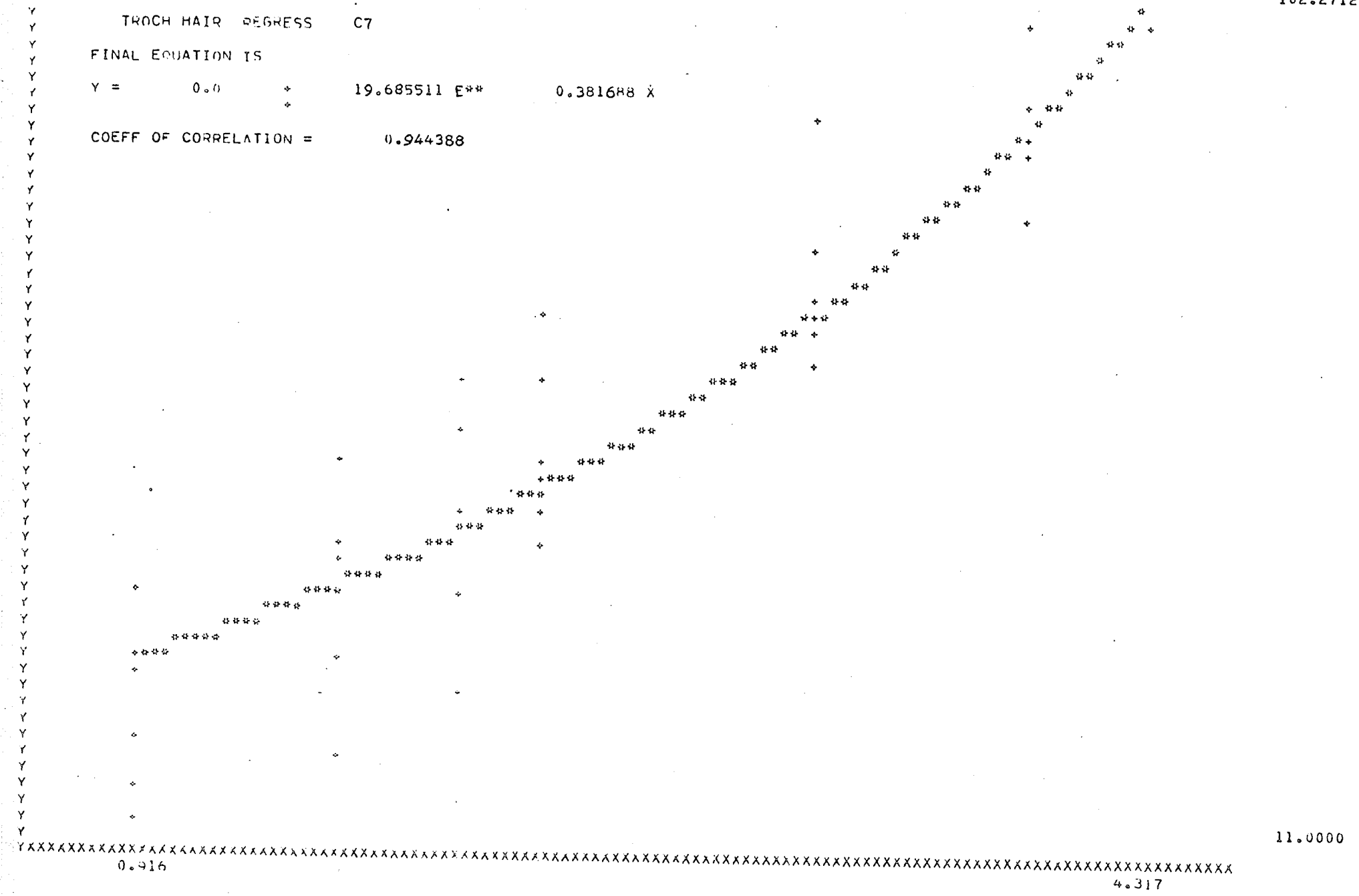

***** UNIVERSITY OF MANITOBA ***** EXPONENTIAL REGRESSION
TROCH HAIR REGRESS
C2 225°
FINAL EQUATION IS
Y = 0.0 + 6.553189 F** 0.597674 X
COEFF OF CORRELATION = 0.964101
102.7441
13.8000
2.303
4.605

FINAL EQUATION IS

Y = 0.0 + 6.684884 E** 0.593856 X

COEFF OF CORRELATION = 0.988719




TROCH HAIR REGRESS C7
FINAL EQUATION IS
Y = 0.0 + 19.685511 E** 0.381688 X
COEFF OF CORRELATION = 0.944388
102.2712
11.0000
0.916
4.317

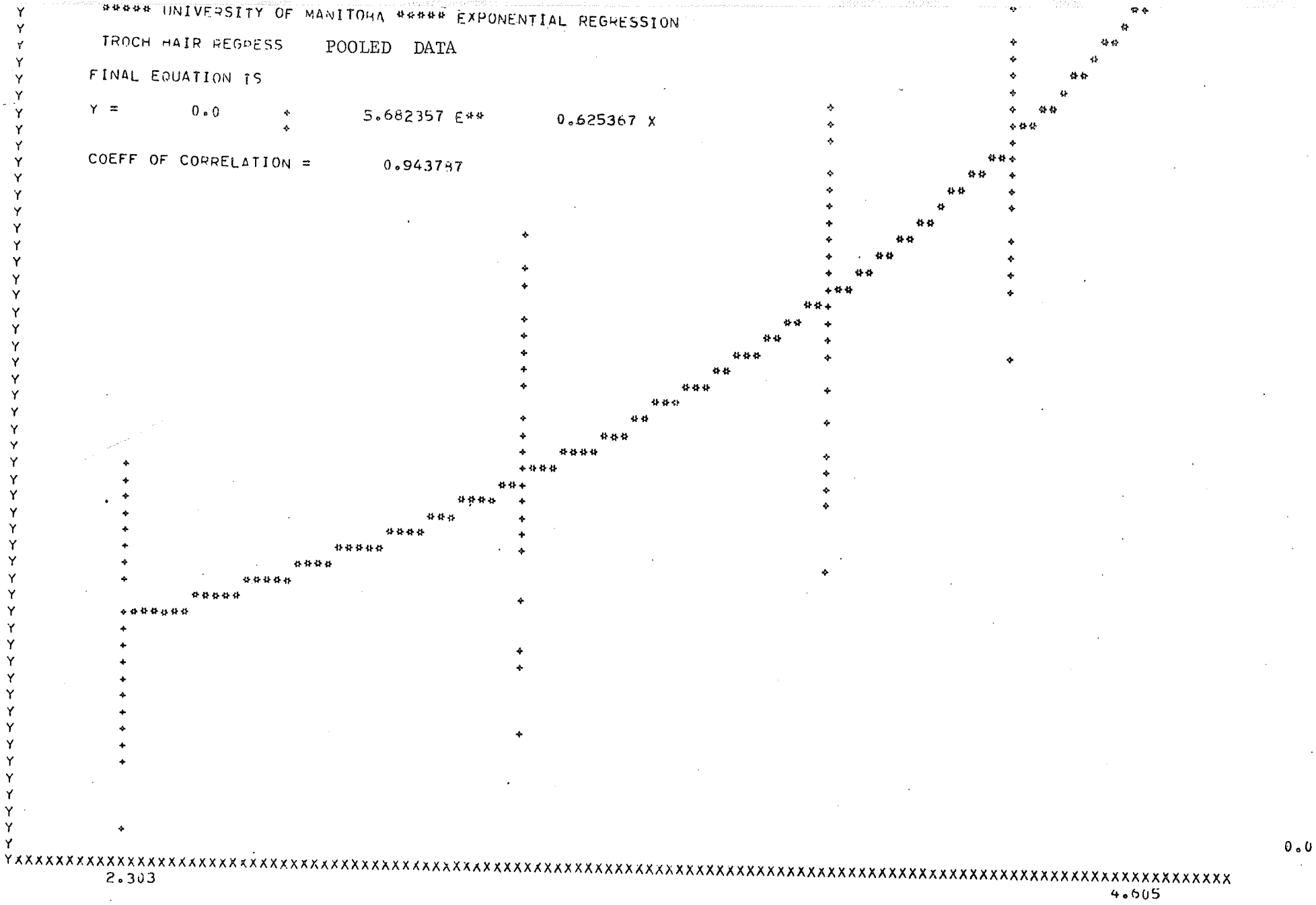
***** UNIVERSITY OF MANITOBA ***** EXPONENTIAL REGRESSION

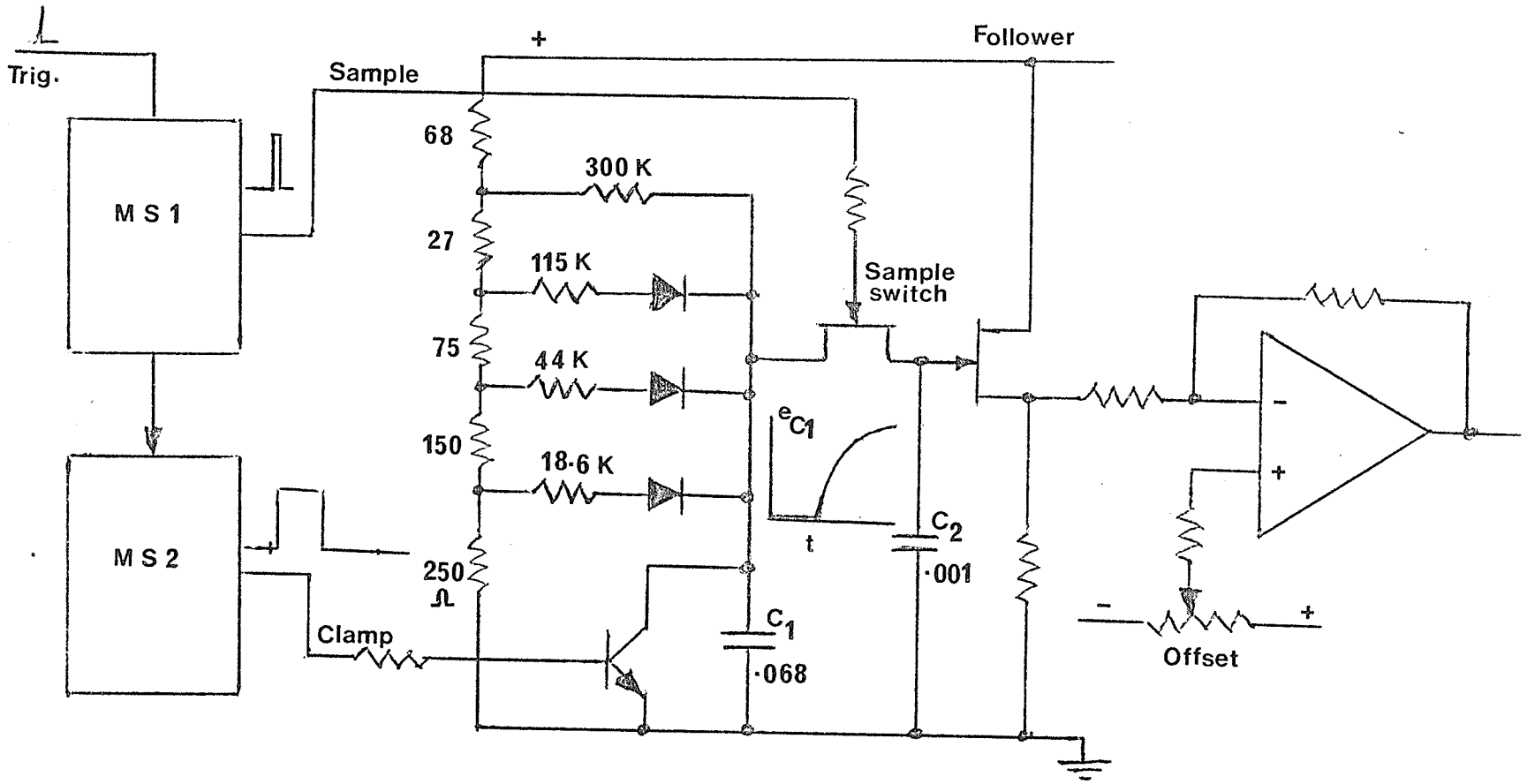
TROCH HAIR REGRESS POOLED DATA

FINAL EQUATION IS

Y = 0.0 + 5.682357 E** 0.625367 X

COEFF OF CORRELATION = 0.943787




Trig.
Sample
+
Follower
MS 1
MS 2
68
27
75
150
250
300 K
115 K
44 K
18·6 K
Sample switch
e_{C1}
t
C_2
·001
C_1
·068
Clamp
Offset
-
+

Appendix 2

Instantaneous Rate Meter

Since the reciprocal of time interval is the instantaneous frequency, a circuit which can perform this reciprocal computation will provide an output which is an instantaneous or beat-by-beat measure of the firing frequency of a pulse train.

Many such circuits have been described and the one used in this study is similar to that described by McDonald (1966).

As the relationship between interval and frequency is a hyperboic function, the circuit performs the computation by forcing the voltage on a capacitor (C_1) to increase in a hyperbolic fashion with time by charging it through a diode shaping network. On the arrival of the next impulse, the voltage on C_1 is sampled and stored on C_2, and the charge on C_1 shorted to ground for the duration of the minimum expected pulse interval (1.34 mS in this circuit). At the end of this period, C_1 is permitted to charge as before. The effect of shunting C_1 to ground for this period is, in effect, to make the rising hyperbolic function cross the 0 volts line at the period equivalent to the maximum frequency to be measured by the rate meter.

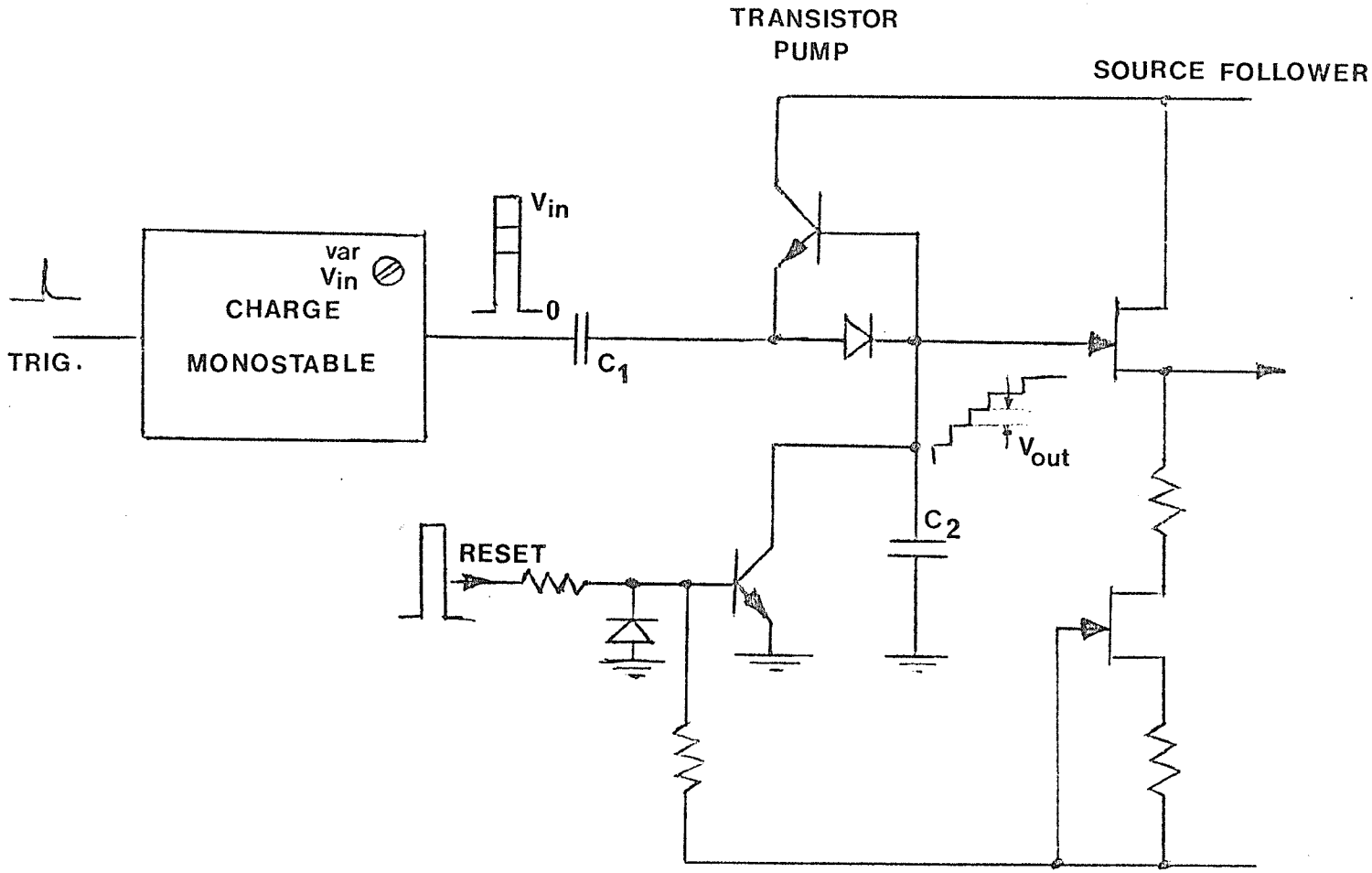
The in coming pulses trigger the sample monostable MS_1, which turns on the FET switch for the duration of the sampling pulse (20 μS) thereby allowing C_2 to charge to the voltage on C_1. The falling edge of this pulse triggers the

Appendix 2 (continued)

Clampmonostable (MS_2), which shorts C_1 to ground via the shunting transistor.

The FET source-follower is used to isolate the charge on C_2 from the operational amplifier which inverts and scales the output voltage from the rate meter such that a 0 to 7.5 V output is obtained for a 0 to 750 pps frequency change.

Ref. MacDonald R. A Heart Rate Meter for Measurement of Instantaneous and Average Rates. Med. and Biol. Engng. 4 291 - 298.


TRANSISTOR
PUMP
SOURCE FOLLOWER
TRIG.
var
V_{in}
CHARGE
MONOSTABLE
V_{in}
0
C_1
V_{out}
C_2
RESET

Appendix 3

Cumulative Pulse Counter (Staircase Generator)

This extremely versatile circuit is based on a transistor pump circuit described by Willis and Burton (1958).

The behaviour of the circuit is given as

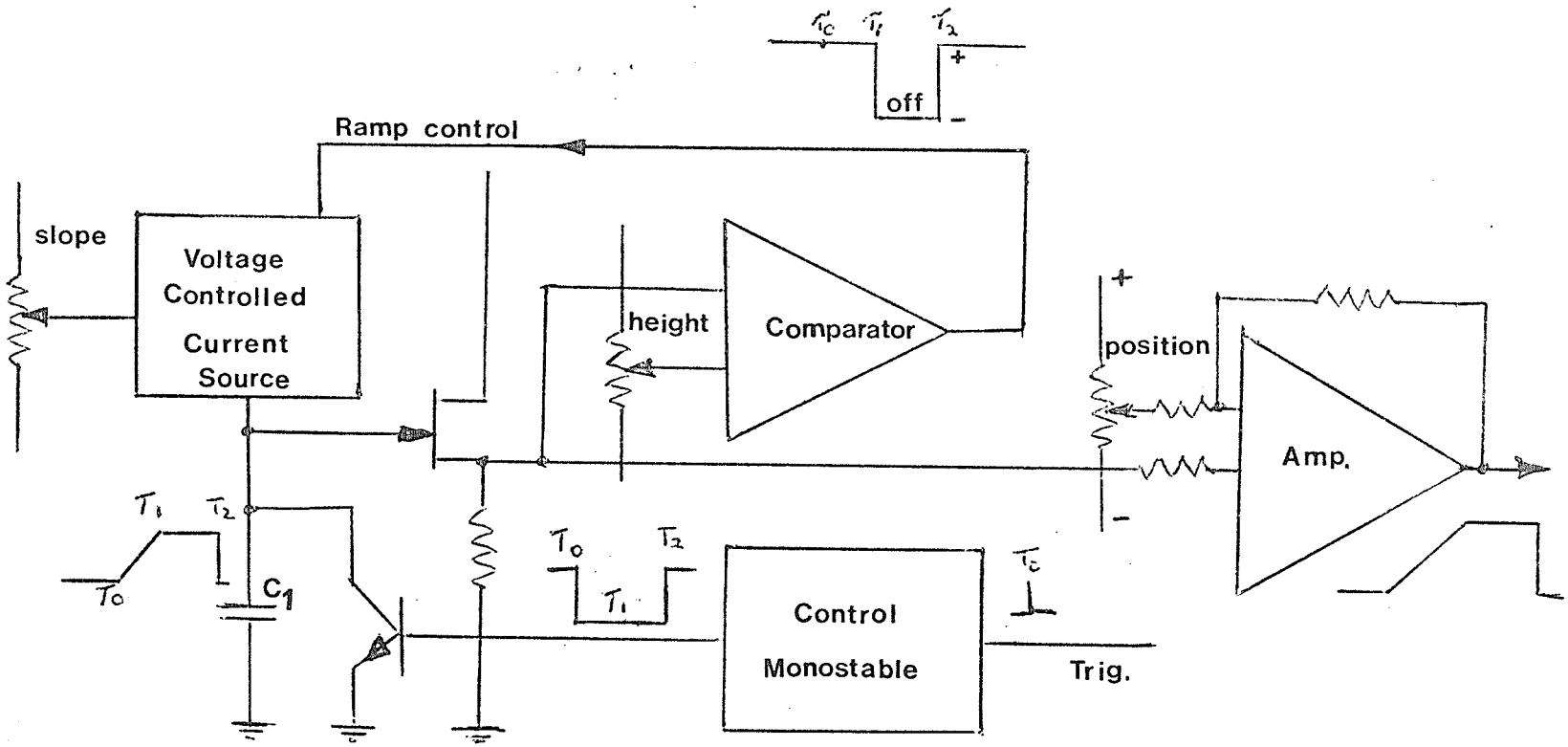
$$V_{out} = \frac{V_{in} C_1}{C_1+C_2} \quad \ldots\ldots\ldots\ldots (1).$$

Essentially the circuit is that of a doide pump, but with the transistor providing isolation between the voltages on C_1 and C_2. As a result, in contrast to the behaviour of the diode pump, the output voltage steps for successive identical input pulse will be equal in height, until the transistor saturates.

Since the connections to C_2 are all extremely high resistance, consisting of a back-biased transistor base-emitter junction, and silicon diode, a biased off shunting transistor and the gate electrode of the field effect (FET) follower amplifier (total $R \simeq 10^3$ Megohm), there is very little droop of the output voltage, provided a low leakage capacitor (polyester) is used. With C_2, a 1 micro-Farad capacitor in the circuit opposite, there was less than 10 mV droop over 1 hour, with a 6 V output voltage.

Since from equation 1 V_{out} is a function of V_{in}, V_{in} is obtained from a separate charge monostable, which in turn is triggered from the signal source. To permit the step height to be varied, the amplitude of V_{in} could be varied.

Resetting the staircase voltage is performed by switching the shunting transistor on by applying a positive pulse to


T_0 T_1 T_2
+
off
−
Ramp control
slope
Voltage
Controlled
Current
Source
height
Comparator
+
position
−
Amp.
T_1 T_2
T_0
C_1
T_0 T_2
T_1
Control
Monostable
T_c
Trig.

Appendix 3 (continued)

the base.

The output circuit is a FET source-follower having a constant current source in place of the source resistance, to permit zeroing the offset voltage inherent in conventional source-followers.

WILLIS, J., and BURTON, P.L. 1958. In Hemmingway, T.K. Electronic Designer's Handbook, B.P. (Lond).

Appendix 4

Ramp Generator

A linear voltage ramp is produced by charging a capacitor through a voltage controlled current source. The current and hence the slope of the ramp is controlled by the voltage obtained from a 10 turn potentiometer, which is calibrated from 0 to 10 millimeters per second. In addition, the ramp slope can be changed in decade steps by changing the value of the capacitor C_1.

In operation, the trigger pulse derived from the external apparatus, triggers the control monostable, which switches off the shunting transistor across C_1. Permitting the current source to charge C_1. When the output voltage of the FET source-follower, which serves to isolate the charge on C_1 from succeeding, is equal to that set by the height potentiometer, the output of the comparator swings negative, switching off the current source. As a result, the voltage on C_1 remains constant at this value until the end of the control monostable pulse is shorted to ground through the shunting transistor.

The duration of the control monostable can be varied, thereby varying the duration of the ramp waveform plateau.

The ramp waveform is amplified by an operational amplifier which also permits fine positioning of the electromechanical stimulator by varying the DC output level.