The stimulus-response relationship, and the structure, of a stretch receptor in the frog's toe

by

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INTRODUCTION.

Introduction.

The mode of action of various sensory receptors has been studied by many workers. Generally a graphic representation has been given of the changes in the discharge frequency, from the receptor, due to a particular stimulation.

The effect of stretch on the discharge frequency of the proprioceptors of the knee joint of the cat has been recorded (Boyd & Roberts, 1952). From study and analysis of the changes in the response frequency, due to the movement, Roberts, Boyd & Cairnie (1956) were able to derive a mathematical relationship between the stimulating movement and the resultant response. By means of this relation - known as the 'transfer function' - it was possible to predict the expected changes in the response frequency due to a specific stimulus.

One such stimulus was a change in the position of the joint with a constant speed of movement. It was found that the alteration in the response, during the movement, was greater than could be accounted for solely by the change of position, and that it was related to the speed of the movement. On cessation of movement the response gradually fell, and, after about 15 sec., had become steady at a value appropriate to the new position. Thus, at any time, the impulse frequency in the afferent nerve from the receptor was

related both to the actual state of deformation of the receptor and to the events which preceded this state.

The impulse frequency, during and after movement, is postulated to be due both to the actual position of the joint and also to some memory of the velocity of the movement by which it reached that position. Thus the transfer function is composed of two parts:

a) That part due to the position, S, of the joint, and so denoted as a function of position, say $\Psi(s)$

$\Psi(s)$ is the displacement component of the response.

b) The part due to some memory of the velocity, v, which is denoted as a function of velocity, say $\Phi(v)$

$\Phi(v)$ is related to the rate of change of position and also to the memory of previous velocities.

Thus the total frequency, which we would expect to observe in the afferent nerve

$$F = \Psi(s) + \Phi(v)$$

In the analysis of the knee-joint proprioceptor responses, it was found necessary to postulate three velocity components in order to obtain a good fit of the experimental curves. One of these velocity components was negative.

Thus -

Total velocity component $\Phi(v) = \Phi_1(v) - \Phi_2(v) + \Phi_3(v)$

and

total response frequency $F = \Psi(s) + \Phi_1(v) - \Phi_2(v) + \Phi_3(v)$

For the purposes of the analysis the frequency component Ψ, due to position, was assumed to be independent of time, i.e. if the frequency due to a certain position is 10 impulses/second when the position is first reached, this component remains at 10 impulses/sec, say 15 minutes later.

The form chosen for the function $\Phi(v)$ was an exponential function. This was based on Katz's (1950b) expression for the depolarisation produced by stretch of a charged membrane. This depolarisation decays exponentially after the stretch. From Katz's work it seems likely that depolarisation at receptor terminals and the discharge frequency in the afferent nerve are directly related.

It would be interesting to see if this transfer function, derived for the stretch receptor in the knee joint of the cat, was of more general application. It seems unlikely that the knee-joint receptor is unique in its mode of generating sensory impulses; the fundamental process involved probably resembles that employed in other stretch receptors. If this transfer function is not just a chance fit, but represents mathematically the action of some underlying process, then it is conceivable that a similar transfer function would be able to predict the response of some other stretch receptor.

The stretch receptor chosen for this confirmatory study was the neuromuscular spindle. The original intention

was to investigate the mammalian spindle using the decerebrate cat. It was proposed to record from the spindle afferents from the tenuissimus muscle, picking up in the dorsal roots, whilst subjecting the muscle to similar types of stretch stimuli as were applied to the knee-joint proprioceptor. Several experiments of this type were attempted with Dr. I.A. Boyd, but were abandoned, largely due to the difficulty experienced in keeping the animals alive for the length of the experiment.

Later work would suggest that one of the reasons for the high mortality may have been that bilateral laminectomy was carried out in all the cats. Unilateral laminectomy would have given an adequate exposure for dorsal root recording and would have entailed much less haemorrhage and subsequent shock. There was also a suspicion that the paraffin used was having a toxic effect on the animal.

A change of experimental animal from cat to frog was therefore made.

Dorsal root recording in the decerebrate frog was attempted. Several difficulties were encountered:-

1) The operative field was very small, thus there was a greater likelihood of damage to fibres.

2) Since the dorsal roots contained so many fewer fibres than, for example, in the cat, they contained a much smaller total number of spindle afferents from any muscle.

Hence damange to any fibres was much more serious than in the cat and it was found to be rather difficult to locate the spindle afferents to a specific muscle.

It was therefore decided that it would be more convenient to work with an isolated muscle-nerve preparation.

The muscle chosen was the frog toe muscle, extensor longus digiti IV, plus its nerve, a branch of the peroneal nerve.

Although this is a very simple preparation which has been extensively used (e.g. by Katz (1949, 1950a,b); Buller, Nicholls & Ström (1952); Eyzaguirre & Vial (1956)), it does not appear to be fully described in the literature. It is described below in some detail.

Among the types of stimulating movement applied to the cat knee-joint proprioceptor were extension at the knee with movements of constant velocity and extension and flexion at the knee with a sinusoidal movement. The frog toe muscle was subjected to similar types of stimulation.

Stretch was applied to the muscle in two different ways.

a) The muscle was stretched from one position to another with a constant rate of change of position.

This type of movement is referred to, throughout this thesis, as a "constant velocity step".

b) It was alternately stretched and relaxed with a sinusoidal variation of position.

The apparatus used for producing these movements of the muscle and for recording the subsequent changes in the afferent discharge is described below, in Part I of this thesis.

After this part of the investigation was completed, a study of the results obtained led to the view that it would be useful to record the effect on the spindle discharge of similar alterations in the tension of the muscle.

Some preliminary experiments of this nature have been done in which step-like alterations in tension have been applied to the muscle.

The relationship between the tension and length of this muscle, during sinusoidal movements, was also determined. A consideration of this relationship gave some indication of the changes which would be expected to result from the use of tension, rather than extension, as the variable stimulus to the muscle.

The apparatus used in these tension experiments is described in Part 1 and the results obtained reported in Part 3.

When the initial experiments were started there was no published histological picture of the spindle system within this muscle. It was felt that it would be valuable to have some idea of the form of the nerve endings within

the muscle. To obtain this a number of muscles were stained with gold chloride and examined. In addition two muscles were stained with haemalum and eosin and serially sectioned throughout their length.

Thus a histological picture of the spindle system within the muscle was built up. The results of this study are given in Part 4.

This histological work was underway when Gray's (1957) study of the spindle and extrafusal innervation of the same muscle, using the methylene blue technique, was published. The results obtained here, though incomplete, give a similar picture of the structure of the spindle.

The results of the analysis in Part 2 of this thesis were presented in a communication to the Physiological Society in September, 1958.

PART I

APPARATUS AND METHODS.

a). Extension experiments.

Preparation.

The dissection was carried out in Frog Ringer. The composition of the Ringer fluid was:-

Na Cl	0.64%
K Cl	0.029%
$CoCl_2$	0.018%
Na HCO_3	0.036%.

The skinned leg was cut off above the knee. On the outer aspect of the knee the peroneal nerve could be seen lying under the tendon of the gastrocnemius muscle (Fig.1A). This tendon (a) was cut and a thread attached to the cut end of the peroneal nerve.

The tendons of the peroneal and tibialis anticus (b) muscles were cut at the knee and the muscles stripped off to the ankle, exposing the peroneal nerve, which branches about halfway down. The medial branch, which does not supply the muscle, was cut.

At the ankle the nerve was carefully freed from the underlying tissues and the foot cut off, just above the ankle.

For the last part of the dissection the foot was fixed to a microscope slide by a rubber band round the toes, and the dissection carried out under a Zeiss binocular dissecting microscope.

The m. ext. longus dig. IV lies over the peroneal artery, which can be clearly seen through the overlying

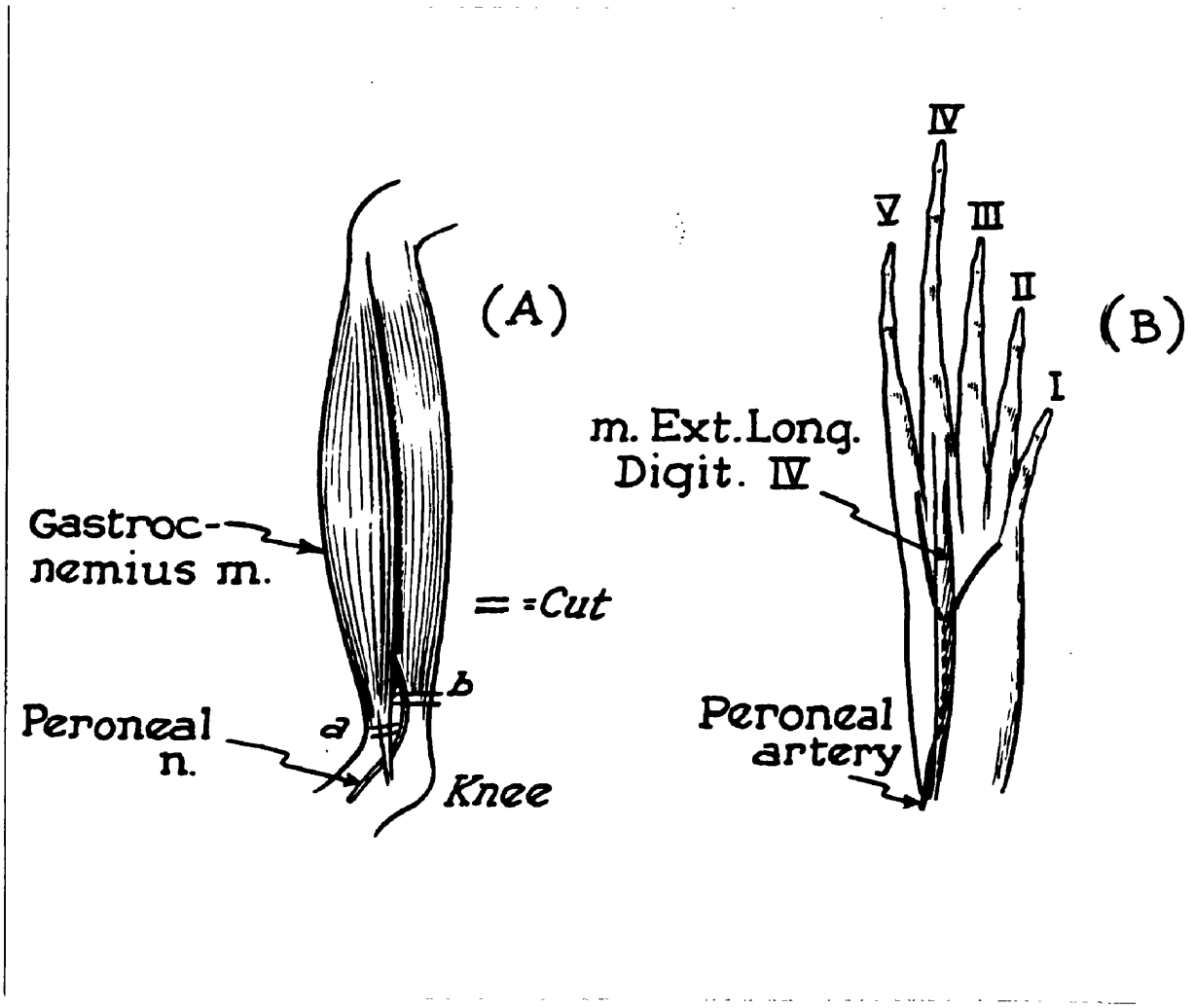


Fig.1. A. Lateral aspect of the lower left leg of the frog, showing the peroneal nerve lying under the tendon of the peroneal muscle (a).

(b) is the tendon of the tibialis anticus muscle.

B. The left foot of the frog showing the position of the m. ext. long. dig. IV, lying over the peroneal artery.

fascia (Fig.1B). This fascia was slit and freed from the underlying tissue exposing the long ribbon-like muscle (Fig.2A).

A large sheet of tendon goes round the outer edge of the ankle. If this tendon is caught at either edge with fine forceps and spread out, the fine thread of the m. ext. long. dig. IV tendon can be seen. This was cut away from the rest of the tendon (care being taken to include any fine separate branches) and a fine nylon thread attached to it.

The muscle was freed from connective tissue and raised gently from the free end. The points of entry of the nerve into the muscle could now be seen (Fig.2B).

The medial and common branches of the nerve were cut, as shown. (The extra portion of the nerve was left as a hold for later clearing.).

The blood vessel was cut below and above the points of crossing of the nerve branches.

The lower nerve branch (a) was not present in every preparation.

Gentle tension was applied to the thread at the end of the nerve and the nerve freed from the underlying blood vessel and connective tissue. The skin branch of the nerve was cut.

The thread at the ankle end of the muscle was raised slightly and the muscle freed up to the tendon at the toe end.

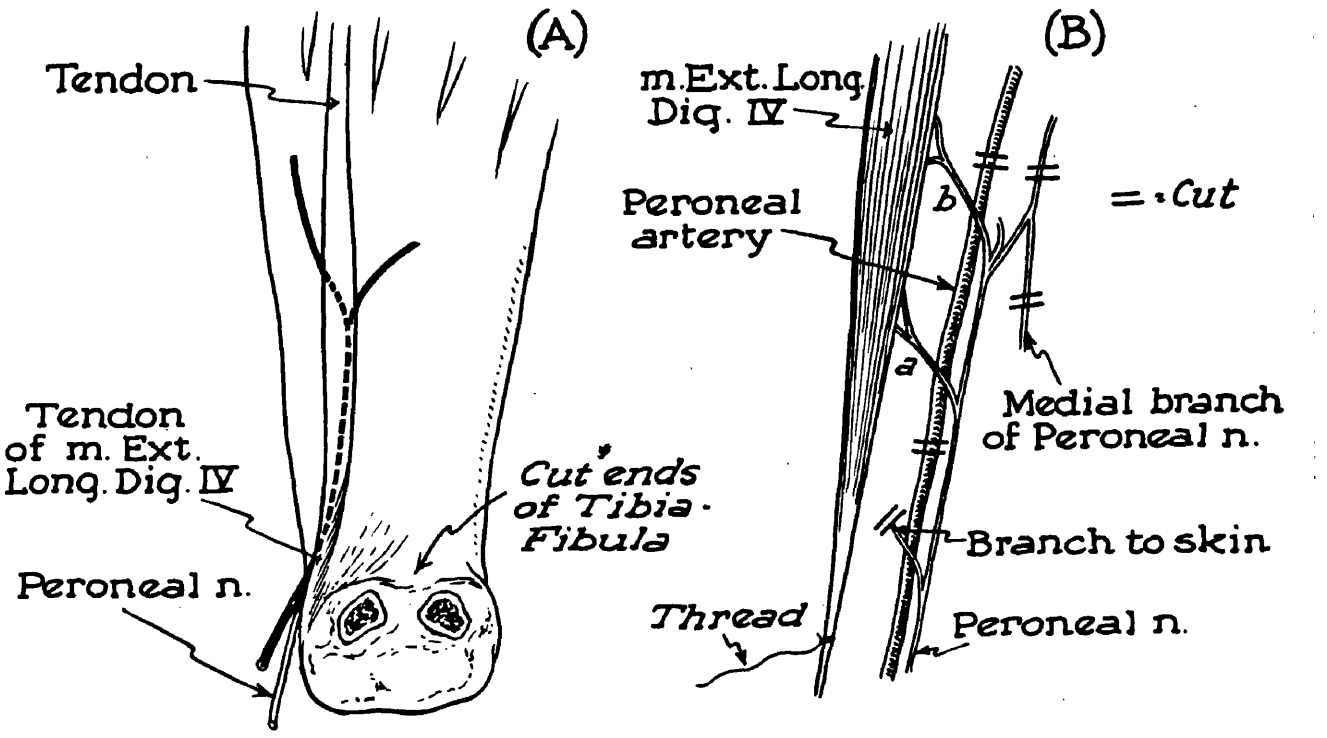


Fig.2. A. M. ext. long. dig. IV showing attachments of tendons.

B. Lower half of the muscle showing the points of nerve entry.

The branch (a) of the nerve is not always present.

This is a sheet like tendon and as it was not very clearly distinguishable the thread was attached before cutting.

This preparation was chosen for several reasons. The dissection presented no particular difficulties, and, although it did not give a single unit response, it was comparatively easy to obtain one by cutting down the nerve. The muscle itself was left intact. The preparation was found to remain in good condition for several hours.

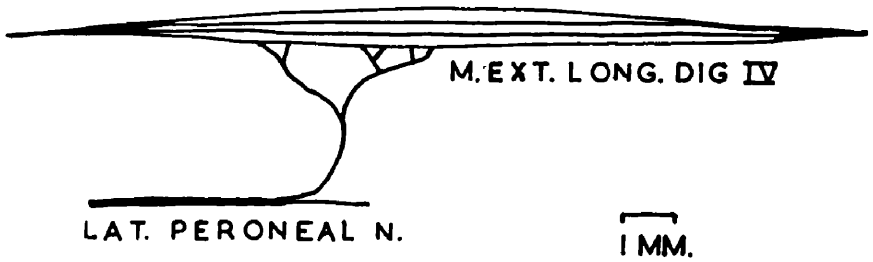


Fig.3. Diagram of m. ext. long. dig. IV and its nerve, showing the arrangement of muscle and nerve which was most commonly found.

"Puller".

The most typical relative positions of the muscle and its nerve are as shown in Fig.3, i.e., the nerve enters the muscle in a region a third to a half way along its length.

Recording from the nerve would be greatly simplified if, on stretching the muscle, the nerve did not move over the electrodes. This stationary position of the nerve could be achieved if the muscle were pulled from both ends, with the point of nerve entry midway between the points of application of stretch. A puller was devised for this purpose by Dr. T.D.M. Roberts. This was constructed as shown.

A movement of, say, 1 cm. of the cord at the point O in Fig.4 leads to an extension of the muscle of approximately 0.4 cm.

The rods AB and BC (Fig.4) were fixed rigidly at right angles to one another. Thus a certain movement of the point A leads to a movement of C which is dependent on the lengths of AB and BC. As C moves, D moves by a similar amount.

The rod DF is pivoted at E so that the movement of the point F is dependent on the length of the arms DE and EF.

The pivots B and E consist of right angled bends of brass sheet connected by overlapping watch springs as shown in Fig.5a.

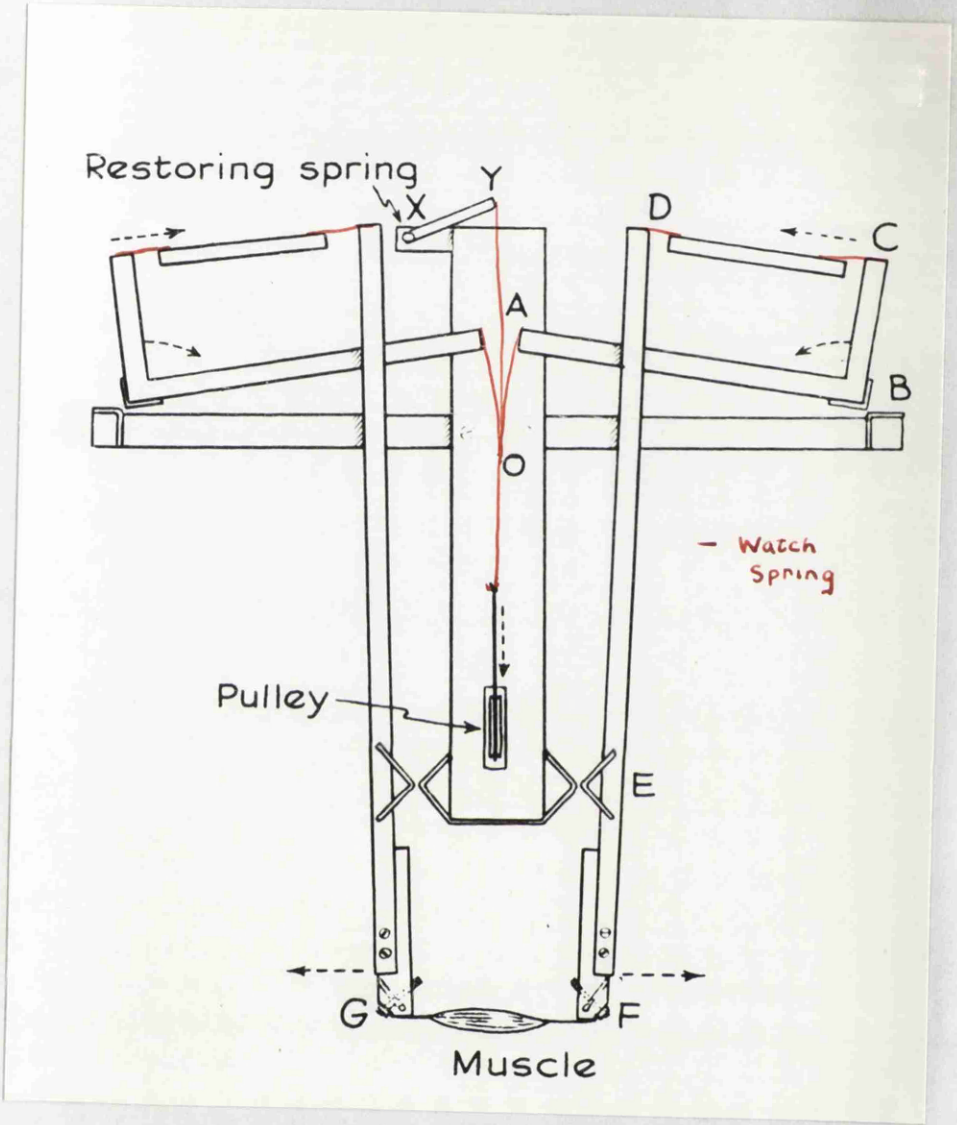


Fig.4. Diagram of the muscle 'puller'.
The muscle is attached to the points G and F.
Tension is applied at the point O.

AB = 9.7 cm. DE = 15.3 cm.
BC = 4.4 cm. EF = 6.5 cm.

When O moves X cm.,
the point F moves $X \cdot \frac{BC}{AB} \cdot \frac{EF}{DE}$ cm.

$= X \cdot \frac{4.4}{9.7} \cdot \frac{6.5}{15.3}$ cm.

$= 0.2\ X$ cm.

Similarly the point G moves 0.2 X cm.
∴ Extension of muscle, corresponding to movement of X cm. at O = 0.4 X cm.

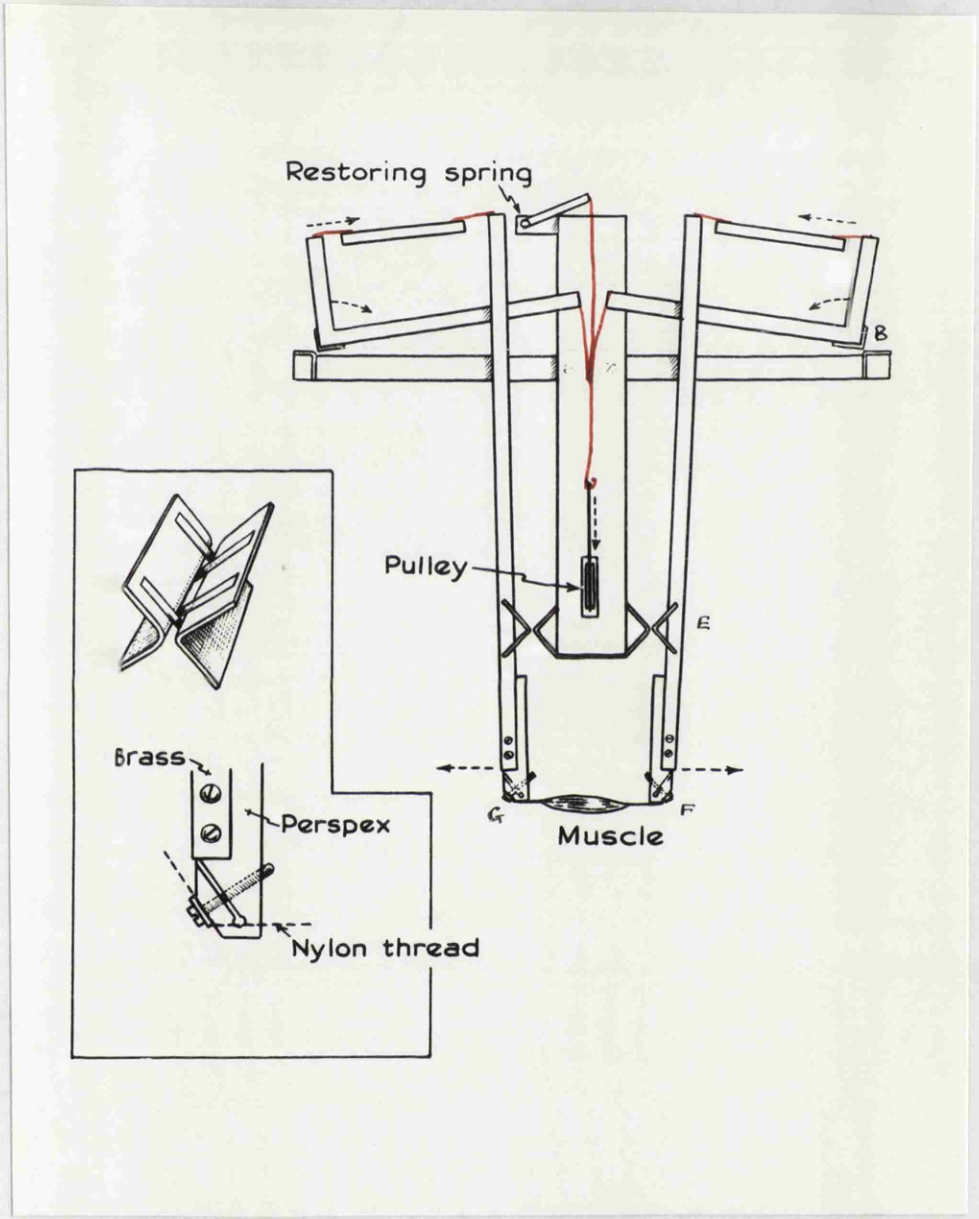


Fig.5. a) Diagram of the type of pivot used at the points B and E.

b) Diagram of the perspex attachment for holding the nylon threads at the points F and G.

The small brass rod XY is connected to a spiral spring at X. When the applied tension is removed from the cord at O this spring restores the arms of the puller to their original position.

At the end of each arm of the puller was a perspex attachment. This is shown in Fig.5b and contained a groove along which the nylon thread from the muscle lay, a binding screw and a perspex washer. Once the screw had been initially adjusted, the thread could be slipped in and out between the perspex surfaces and held with an appropriate tension.

The position of the muscle was arranged so that the point of nerve entry was approximately midway between the ends of the puller. The tension was adjusted so that the muscle was just not relaxed.

The cord from the puller was attached via a system of pulleys to either of the stretching devices described below.

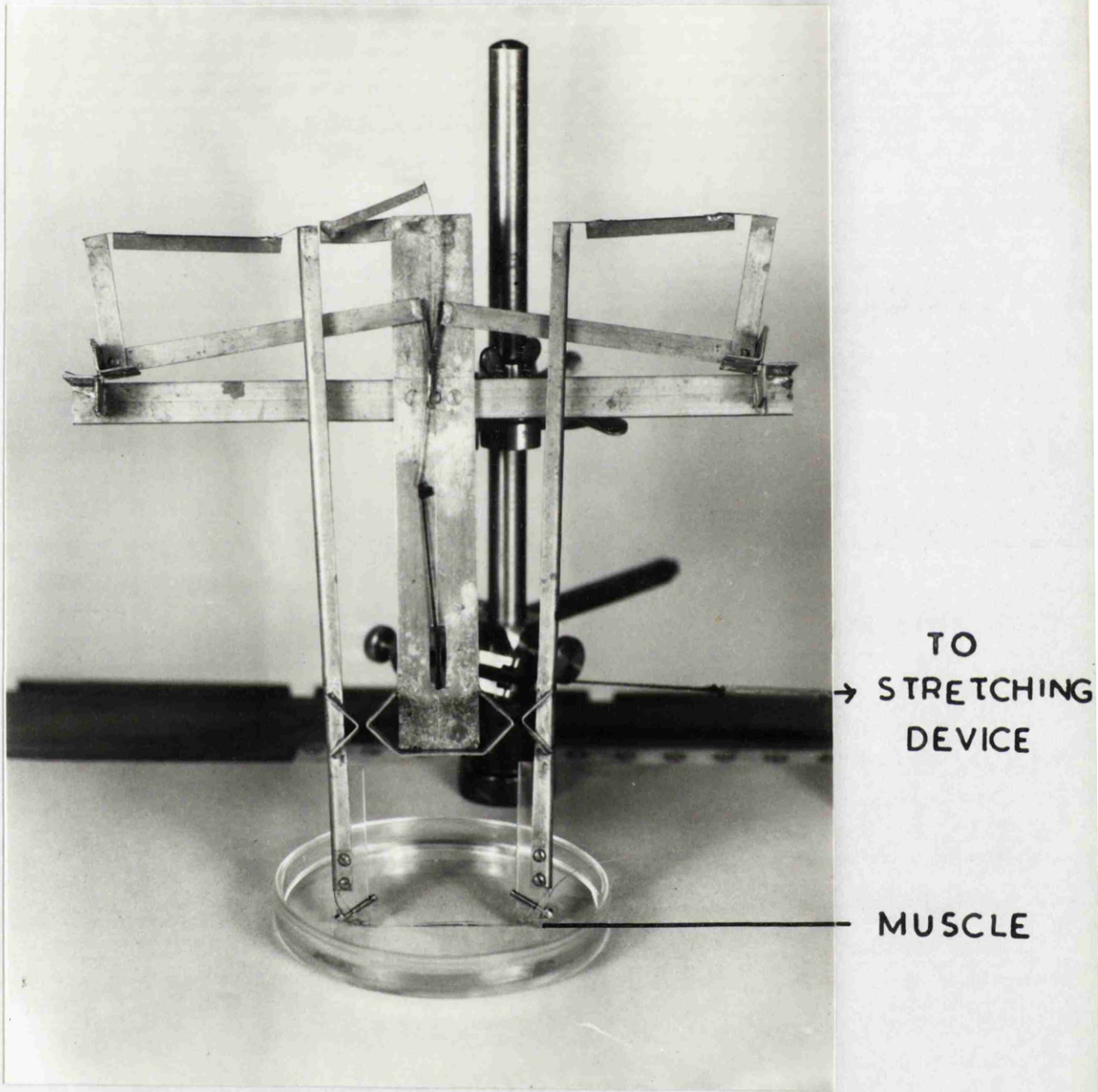


Fig.6. Photograph of the 'puller' with a muscle in position.

Stretching Devices.

The types of stimulating movement applied to the muscle were:-

a) Extension of the muscle with a constant rate of stretch, i.e., constant velocity steps.

b) Extension and relaxation of the muscle with a sinusoidal movement.

These movements were produced as described below.

a) Constant velocity steps.

The cord from the 'puller' was attached to a system of pulleys which were driven by the fall of a dashpot. This dashpot fell at a steady rate which could be varied by means of a screw control which moved up and down on a calibrated scale. Figure 7 shows a photograph of the dashpot system.

The extent of the stretch applied to the muscle was altered by varying the size of pulley driven by the dashpot.

The positions of the screw control which covered the widest range of rates of fall of the dashpot were determined. The same positions were used in different experiments.

b) Sinusoidal movements.

The cord was attached to a beam driven by an electric motor. This beam was pivoted at one end and moved vertically with a sinusoidal movement.

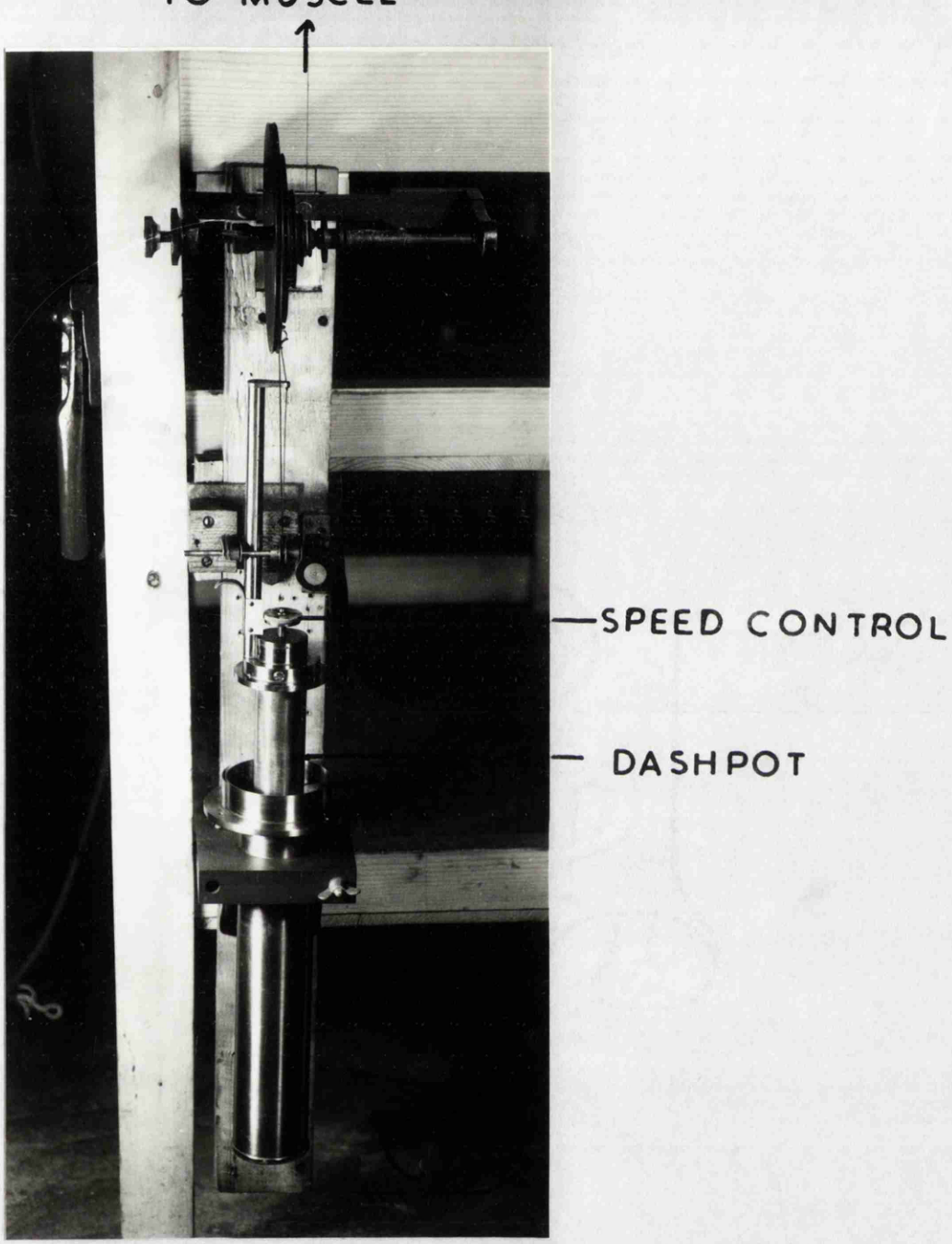


Fig.7. The dashpot system which was used to apply 'constant velocity steps' to the muscle. Fall of the dashpot at a controlled rate rotated the pulleys to which the cord from the puller was attached.

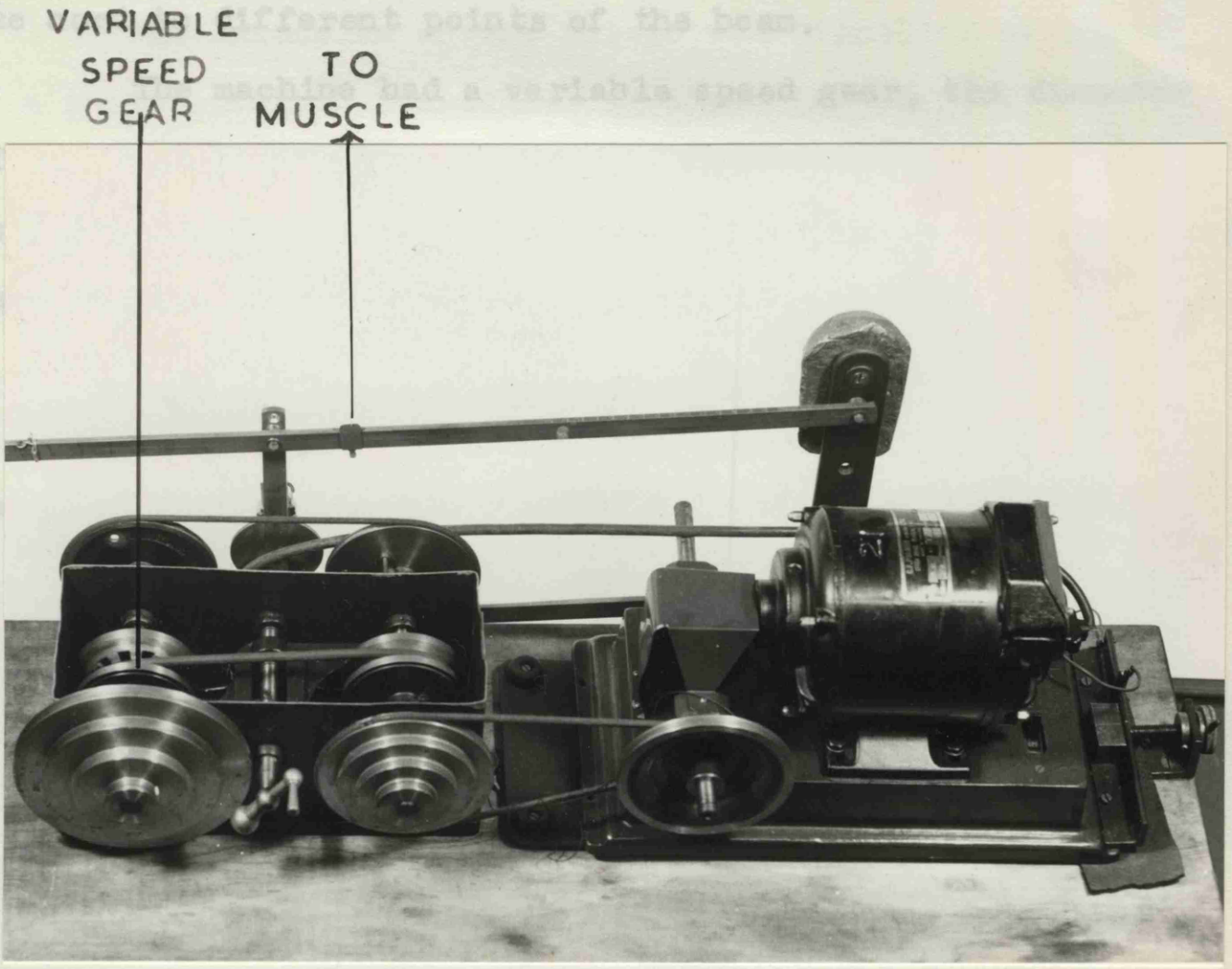


Fig.8. Photograph of the machine used to produce the sinusoidal movements showing the interlocking toothed pulleys whose diameter could be altered by means of the crank, thus altering the speed of the movement of the pivoted beam.

The extent of the movement was altered by attaching the cord to different points of the beam.

The machine had a variable speed gear; the diameter of the interlocking toothed pulleys round which one driving belt ran could be altered by means of a crank. It was found that if the crank was wound a) fully in,

b) midway,

c) fully out,

rates of movement in the ratio 2:3:4 could be obtained.

A photograph of this machine is shown in Figure 8.

Movement Signal.

The extension of the muscle was signalled by means of a photo-electric transducer as shown in Figure 9.

The cord joining the puller to either of the stretching devices was attached, en route, to the long arm of a freely pivoted lever. The other arm of the lever was a paper flag which moved between a light source and two adjacent photo-electric cells. Movement of the flag led to an alteration in the output from the cells. This output was led into channel 2 of the amplifier.

A millimetre scale was placed behind the point of attachment of the lever to the cord. This scale was used to indicate position and extension during the experiment. By means of this scale it was possible during the experiment to restore the muscle to any desired position.

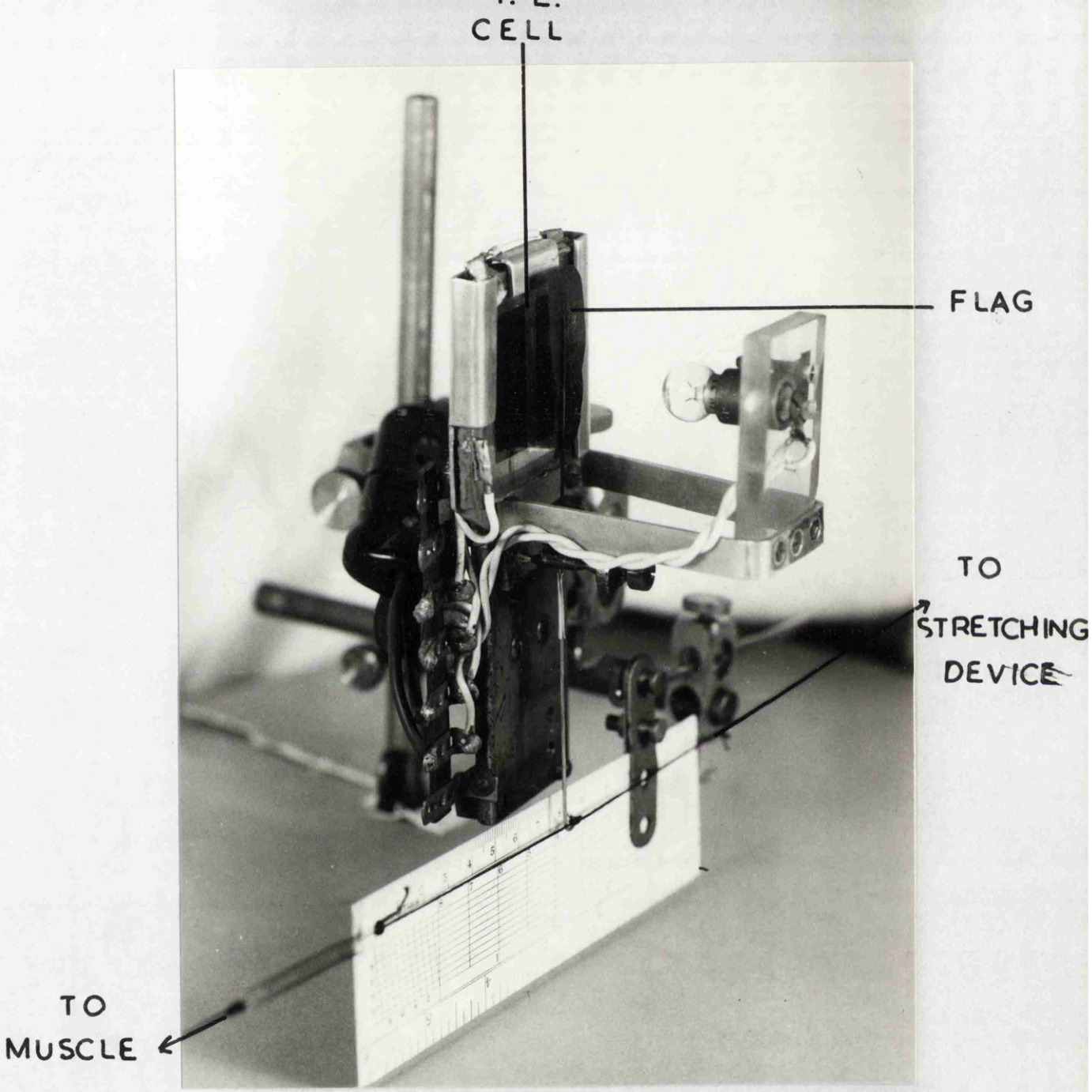


Fig.9. The photo-electric transducer which was used to measure the length changes applied to the muscle. The long arm of the freely pivoted flag was attached to the cord joining the 'puller' to either of the stretching devices. The flag moved between a light source and two adjacent p.e. cells. Movement of the flag altered the output from the cells, which was fed into channel 2 of the oscilloscope. The transducer was enclosed in a shield of black paper.

The millimetre scale used to indicate position is also shown.

Recording Apparatus.

These experiments were conducted inside a screening cage of fine mesh wire netting.

A block diagram of the apparatus used in recording the discharge in the afferent nerve, during stretch of the muscle, is shown in Figure 10.

The electrodes used were Pt electrodes (Fig.11). Pt wires were welded to copper wires and then fused inside glass tubing, leaving about 2 cm. Pt wire exposed. The electrodes were shielded from tips to input cable; first by brass tubing, then by wire screening. This screening could be connected to earth if necessary.

Wander plugs were fitted to the ends of the short electrode leads and connected to the input cable of the pre-amplifier. The output from the pre-amplifier was fed into a loud speaker unit, one beam of the amplifier and display unit and also into a pulse interval meter. The output from the pulse interval meter was then fed into a moving coil meter which was calibrated to give a direct reading of the frequency of the impulses from the pre-amplifier.

The second channel of the display tube was used to record the movement signal from the photo-electric transducer. This beam also carried the time marker which was derived from a clockwork system giving pulses at $\frac{1}{2}$ sec. intervals.

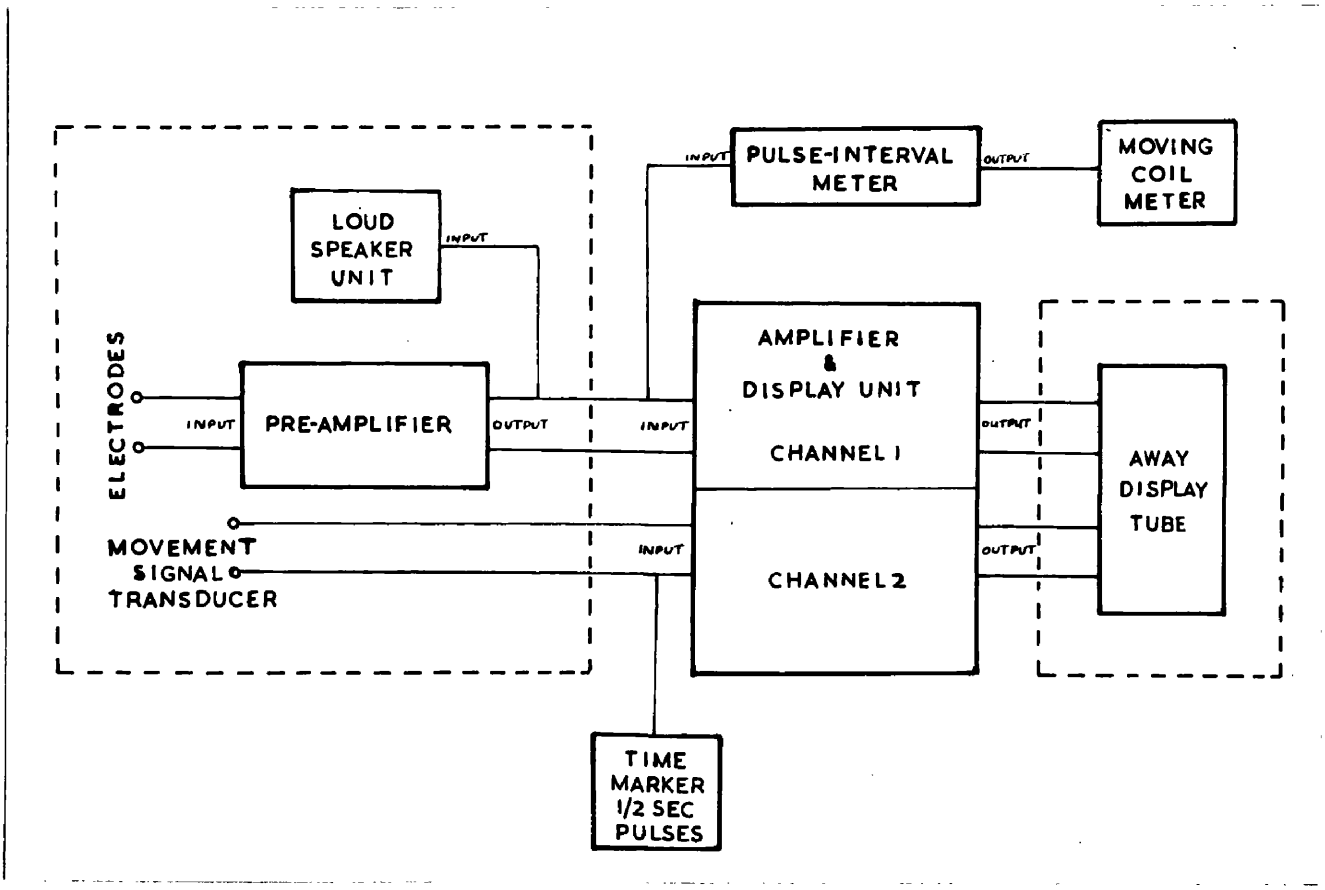


Fig.10. Block diagram of the recording apparatus. Those pieces of equipment inside the dotted lines were kept within the screening cage.

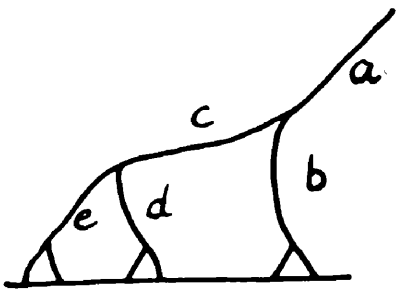
Fig.11. The Pt electrodes used for picking up the sensory discharge.

A Cossor camera was mounted in front of the oscilloscope tube of the display unit for photographic recording of the movement and the response.

Single Unit Response.

When the dissection of the muscle-nerve preparation was completed it was placed in fresh Ringer solution in a Petri dish. The nylon threads, attached to the tendons of the muscle, were placed in the grooves at the end of the 'puller' and over the binding screws. The threads were adjusted so that the nerve was midway between the arms of the 'puller', and the muscle was neither stretched nor relaxed. (This tension adjustment was done by eye; no check was made with the resting length in situ).

A layer of liquid paraffin was poured over the Ringer solution in the Petri dish. The recording of the sensory impulses in the nerve was monophasic. One electrode was placed in Ringer, the earth electrode; the other electrode, over which the nerve was placed, was in liquid paraffin.



The diagram shows the most common mode of innervation of the muscle.

The main branches of the nerve were placed in turn over the recording electrode, using small black glass rods. In no case was a single unit response obtained at this stage.

The response might be:-

	No. of units
Recording in branch a	5
b	3
c	4
d	2
e	2

The branch with the fewest units and of the most convenient length was selected. Its entry into the muscle was cleared. Sometimes at this stage a 'single unit' response could be obtained by stroking the nerve with closed watchmakers' forceps even though, say, 3 fibres could still be seen. On other occasions, cutting had to be continued until only one branch remained. Usually it was a matter of chance whether this branch was a sensory one, but on a few occasions it was possible to place the electrode under the very short terminal nerve branches and see which gave a discharge on stretching the muscle.

The instruments used in the dissection are shown in Figure 12.

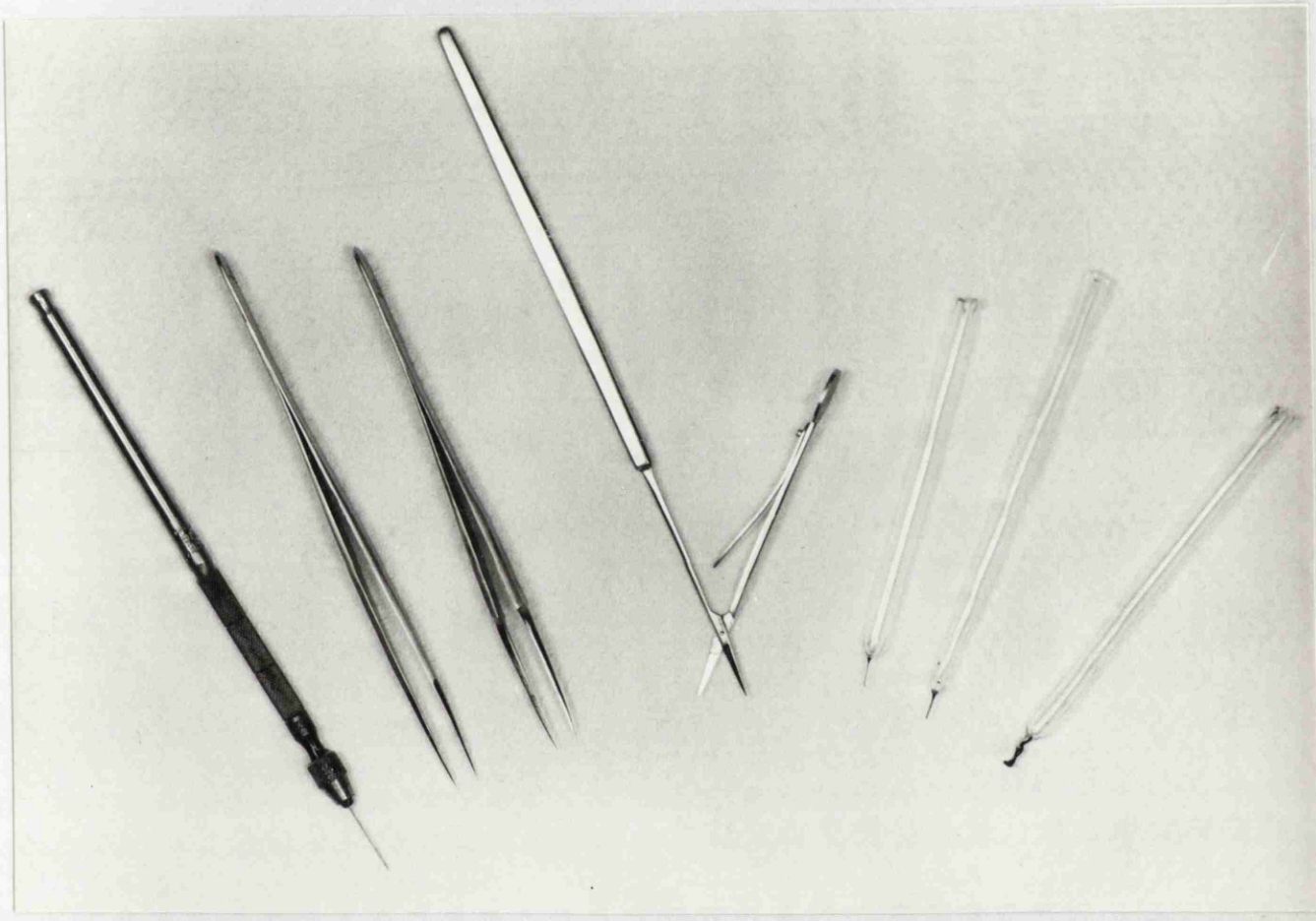


Fig.12. Some of the instruments used in the dissection.

b). Tension experiments.

Preparation.

In both sets of experiments on the application of tension to the muscle, it was dissected out in the same manner as in the extension experiments. The only difference was that in the experimental determination of the length/tension relationship, since the nerve was not required, it was severed at its point of final branching.

The muscle was placed in Ringer fluid in a bath (described below). For nerve recording a layer of liquid paraffin was placed over the Ringer.

Muscle Bath.

In these tension experiments, since the muscle was being stretched much more violently than previously, it was felt that it would be desirable to change the Ringer fluid during the experiment.

A special perspex bath was made to facilitate this change. The bath, with its communicating side chamber, is shown in Figure 13. Ringer fluid flowed into the main bath from a constant pressure burette, through a polythene tube. A fine pointed tube dipped into the side bath. This tube was connected to a water suction pump, via a glass jar which acted as a water trap. When the fluid level in the side bath rose to the level of the tube, the suction pump came into action. Thus the level of Ringer in the main bath could be

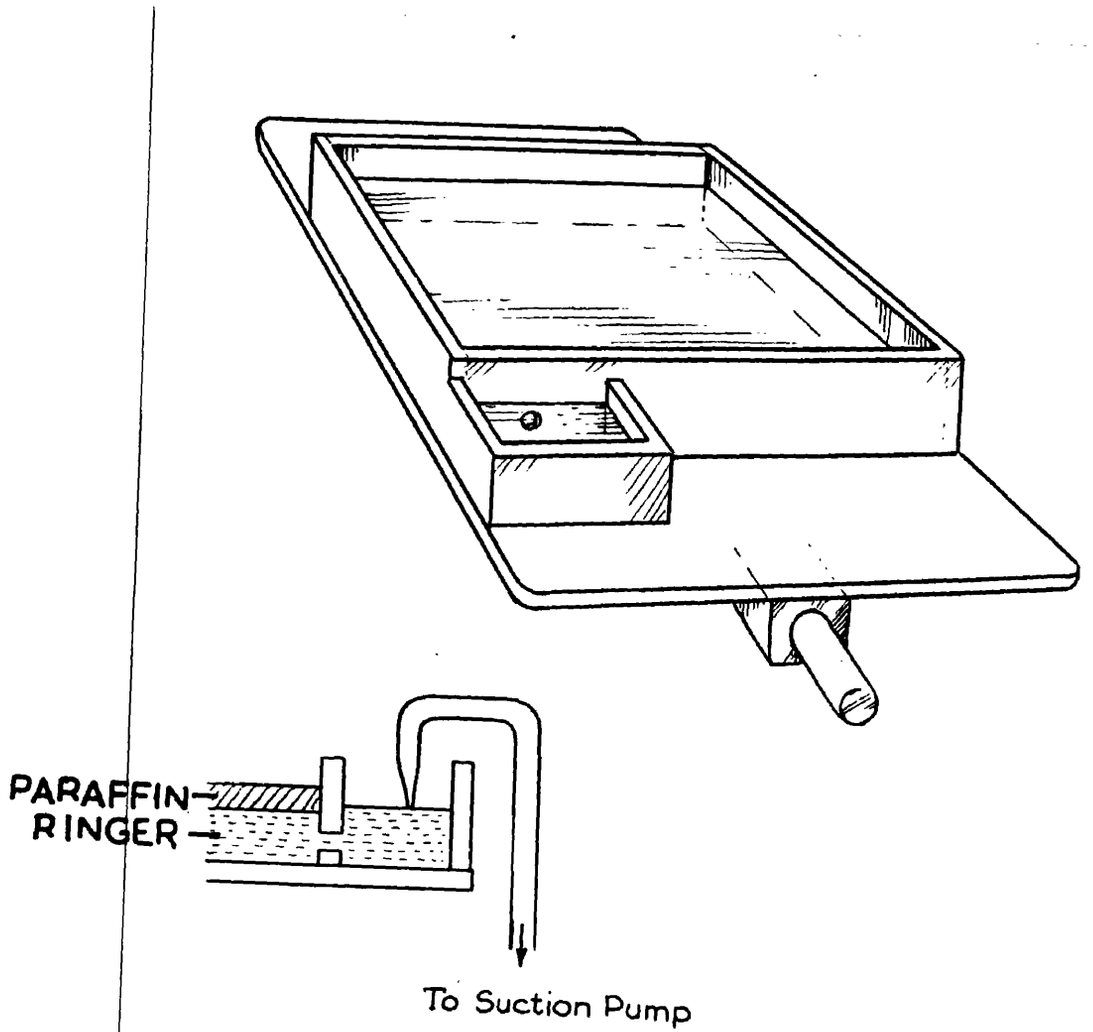


Fig.13. Diagram of the perspex bath showing the communicating side chamber.

kept at a pre-set value. This level was such that the muscle and the earthing electrode were in Ringer while the recording electrode was in the superimposed paraffin layer. In practice, it was found that, due to fluctuations in the water pressure, the suction pump did not always come into operation when the fluid level rose. The system was therefore used for occasional, rather than continuous, changing of the Ringer fluid.

A brass strip was fixed inside the bath to enable the fluid to be earthed easily.

This bath had the advantage that it was more secure than the Petri dish used previously and it could be fixed in any desired position.

Lay Out.

After dissection, the muscle was placed in Ringer solution in the bath, and a nylon thread attached to each end. The loop at the end of each thread was passed round a hook on a light perspex lever. The experimental arrangement is illustrated in Fig.14. One lever was attached to the moving coil system which applied the tension to the muscle and the other to the tension transducer.

Fig.15 gives a diagram of the perspex lever. These levers were freely pivoted at one end. At the other end, which dipped into the Ringer in the bath, the levers were slotted for attachment of the loops of nylon thread. On each lever, 0.5 inches from the slotted end, a light wire hook was passed through a hole in the perspex. To these hooks were attached threads going to either the system for recording or for application of tension.

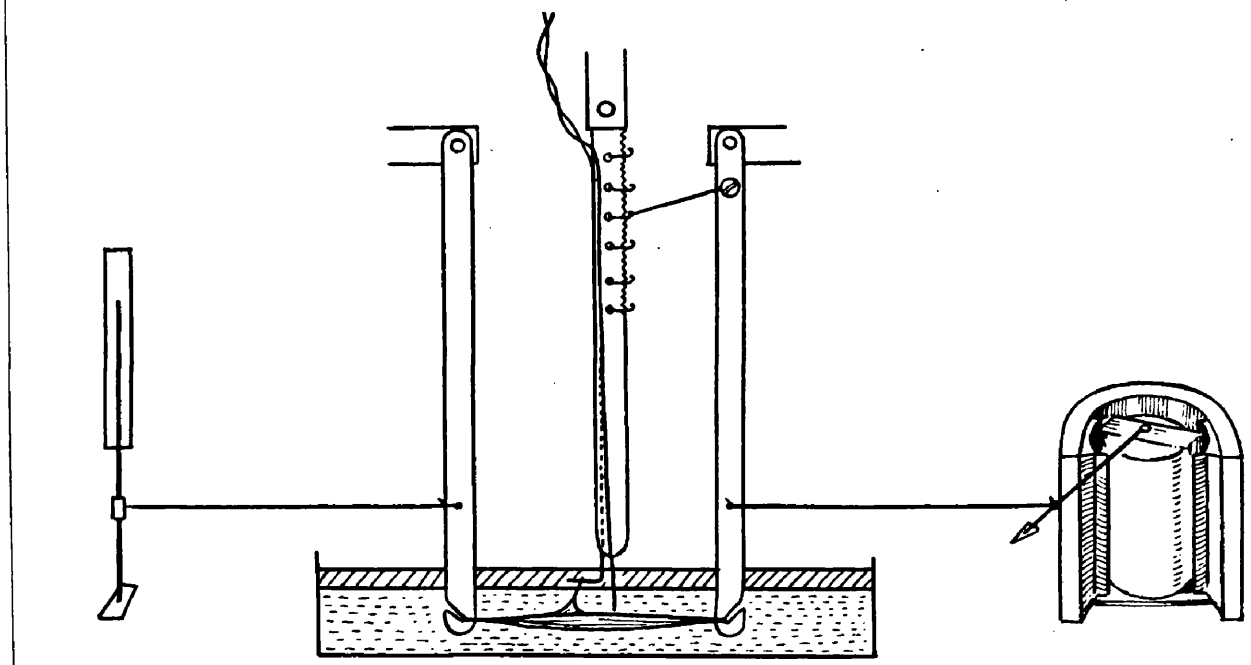


Fig.14. The experimental lay-out for recording the effect of the application of tension "steps" on the sensory discharge. One lever is connected to the moving coil system for the application of the tension and the other to the tension transducer.

The nerve lies over the recording electrode in the layer of liquid paraffin while the muscle and the earthing electrode remain in Ringer.

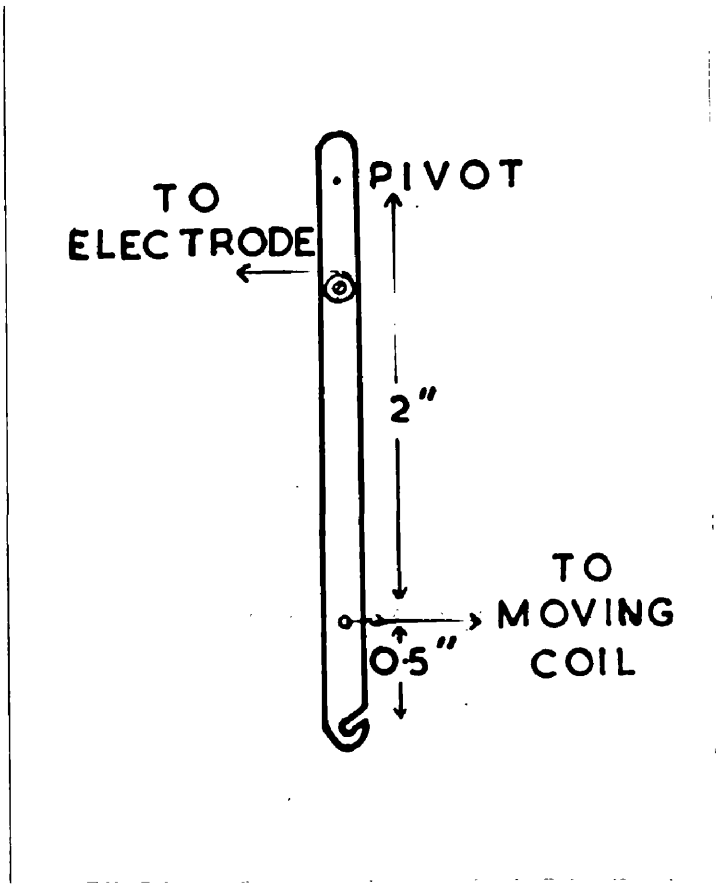


Fig.15. Diagram of one of the perspex levers to which the muscle was attached. This lever had a perspex washer screwed into it near the pivot. A thread from the electrodes was passed between this washer and the lever.

Application of Tension.

Tension was applied to the muscle by means of a moving coil system. This coil had a brass rod attached at right angles to it. The rod could be connected, by a thread, to the perspex lever.

The force exerted by the coil was directly proportional to the current flowing through it. In the initial experiments this current was not continuously variable, so that the application of tension occurred suddenly (on completion of the coil circuit) and the only variable was the final tension. In the length/tension experiments the current in the coil circuit could be varied as desired, and thus varying tensions could be applied to the muscle.

a) 'Steps'.

The coil was connected, in series, with a battery, a fixed resistance, a potentiometer, and a key, the closure of which caused the sudden application of tension. The voltage of the battery and the resistance of the potentiometer could take different values, thus altering the current through the coil and the tension produced by it. The tensions produced by various different values of resistance and voltage were measured by means of a torsion balance.

b) Length/Tension Determination.

In the later experiments, on the relationship between

length and tension for this muscle, it was possible to vary the current passing through the coil and thus apply a variable tension to the muscle. This was done by using a resistance bridge method suggested by Dr. T.D.M. Roberts. The circuit is shown in Figure 16. The current passing through the coil could be altered by varying the 50 K potentiometer. This potentiometer was attached to a pulley. The pulley could be rotated by either of the systems used to stretch the muscle in the extension experiments, i.e., the dashpot system, for constant velocity steps, or the S.H.M. machine, for sinusoidal variations. Thus the tension in the muscle could be varied either sinusoidally or with constant rate of change of tension.

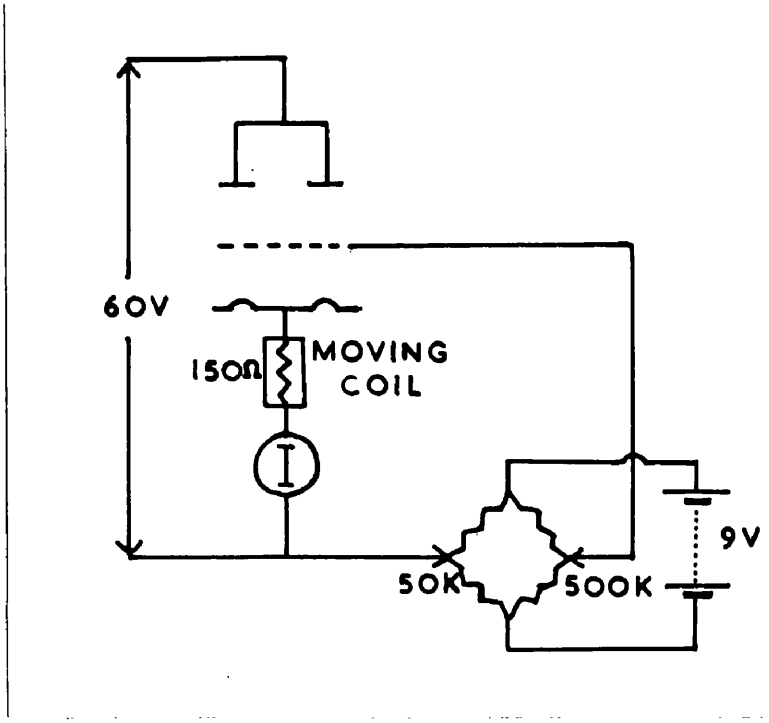


Fig.16. Circuit used for the application of varying currents to the moving coil. The 50 K potentiometer was attached to a pulley which was connected to either the dashpot system or the S.H.M. machine. Thus the current in the coil could be varied either with a constant rate of change or sinusoidally.

Recording of Tension.

The tension applied to the muscle was measured by means of a photo-electric transducer similar to that used for signalling movement in the extension experiments.

The application of tension caused a flag to move between a light source and a photo-electric cell. The output from the p.e. cell is dependent on the amount of light falling on each side of it. Movement of the flag altered this and thus signalled the tension.

The arrangement is shown in Fig.17. The transducer was contained in an aluminium box, the thread from the lever passing in through a slit in the lid.

The paper flag was attached to a light aluminium tube which was fitted over a thin steel rod soldered to a watch spring. The watch spring was firmly screwed down at both ends. The thread from the lever was attached to a hook which slid over the aluminium tube. The hook could be fixed to any point on the tubing, thus altering the excursion of the flag produced by a particular tension. It was usually placed near the base of the rod so that the actual movement of the point of attachment was very small.

The output from the transducer was too unstable to measure the absolute values of the tensions applied. This was done by means of a torsion balance. It was however possible to measure the increase in the tension during the

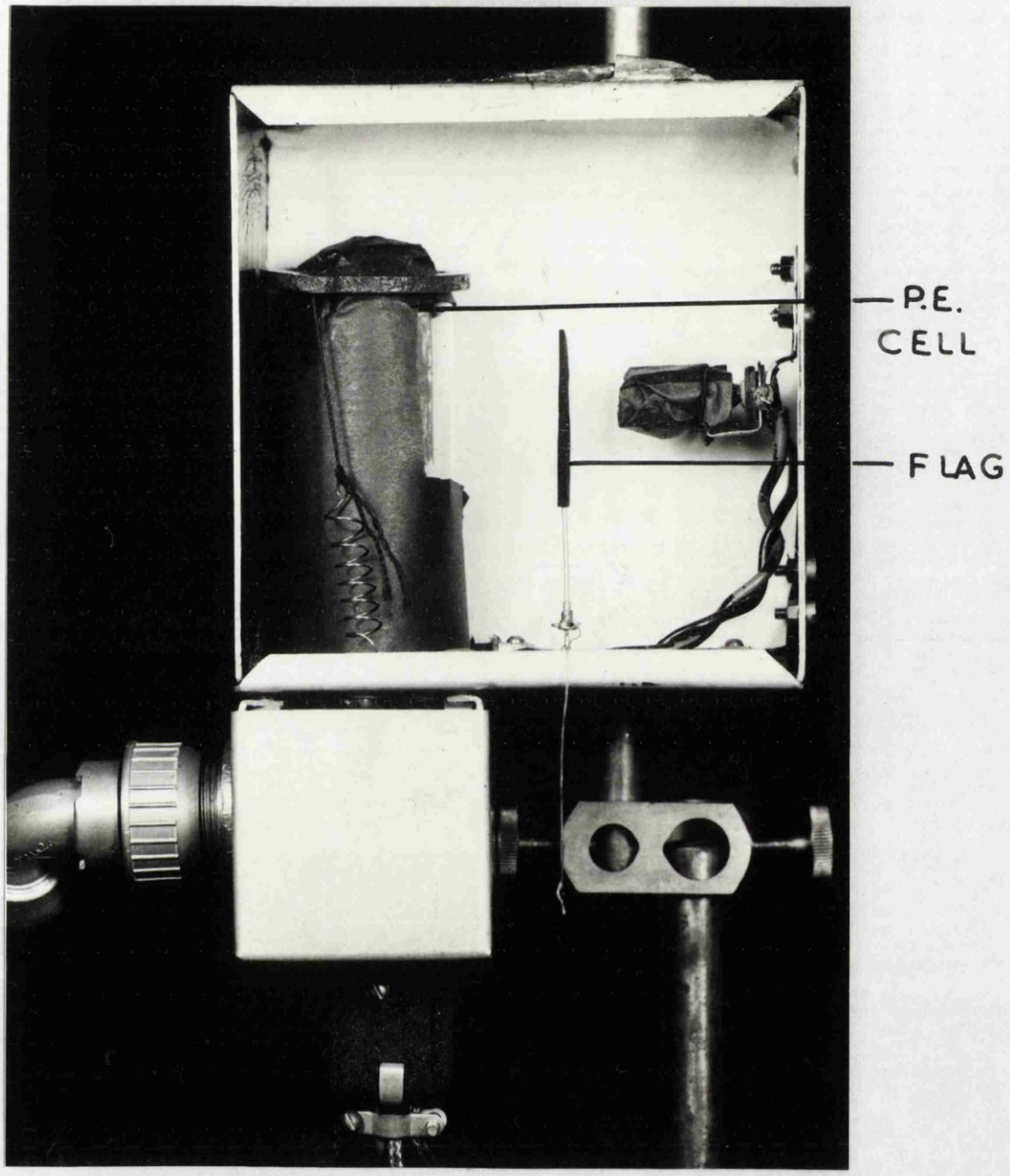


Fig.17. The photo-electric transducer used to measure the changes in the tension applied to the muscle. The flag, to which the thread from the muscle lever is attached, moves between a light source and a p.e. cell. The light bulb was hooded with a central slit, in order to cut out reflections from the inside of the aluminium box which was painted black.

movement.

After amplification, by means of a double triode, the output from the transducer was fed into either a cathode-ray oscilloscope or a pen recorder.

Glycerine Transducer.

In the determination of the length/tension relationship for this muscle the alterations in length were measured by means of this transducer.

Each of the levers to which the muscle was attached carried a perspex side arm. Through a hole at the end of each side arm passed a piece of silver wire which dipped into a perspex bath containing a glycerine and saline mixture (Fig.18).

These wires were connected to a fixed resistance and to a potentiometer to form one arm of a resistance bridge as shown (Fig.19). Saline was added to the glycerine in the bath to lower the resistance of the fluid.

Fine enamelled wire was used to connect the silver wires to the potentiometer so that there would be no hindrance of the movement of the levers. The silver wires moved along with the levers and thus the resistance of that arm of the bridge was altered.

The output from the bridge was fed into the pen recorder.

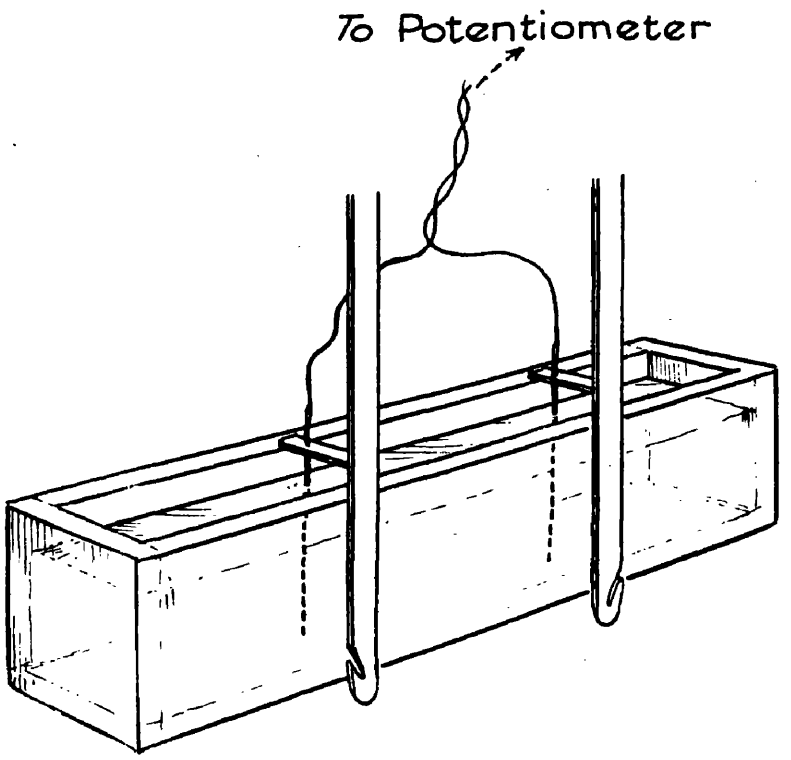


Fig.18. The glycerine transducer used to measure the changes of length in the muscle on the application of varying tensions. Silver wires dipped into a glycerine and saline mixture in a narrow perspex bath. Movement of the levers altered the resistance between the silver wires. This resistance formed one arm of a resistance bridge (Fig.19) and thus the length changes were signalled.

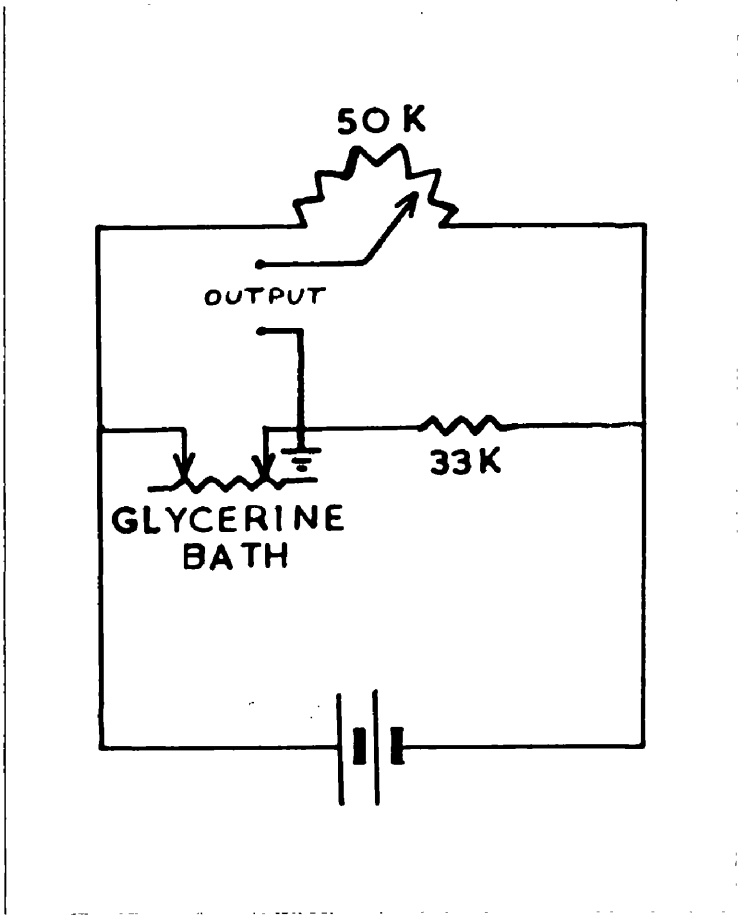


Fig.19. Diagram of the circuit of the resistance bridge arrangement used to measure the length changes in the muscle produced by the application of varying tensions. The silver wires attached to the perspex levers dip into the glycerine bath. Movement of the levers alters the resistance of one arm of the bridge and thus the change in length is signalled.

Electrodes.

When the tension steps were applied to the muscle it was considerably stretched and the point of nerve entry moved an appreciable distance. If it was attempted to record the effect of the application of tension on the spindle discharge using stationary electrodes, the nerve either broke, came off the electrodes, or moved over the electrodes to such an extent that a large movement artefact was recorded. To overcome this difficulty movable electrodes were made.

The most satisfactory recording conditions were obtained when the electrodes were attached to a lever which could be linked to the other levers. Thus the electrodes could move in conjunction with the nerve.

The electrodes consisted of Pt wires soldered on to fine enamelled wire and attached to a thin metal strip. This metal strip was freely pivoted at one end and had a number of small holes punched in it to which small wire hooks were attached.

One of the perspex levers had a perspex washer screwed into it near the pivot. A fine thread could be attached to any of the hooks in the electrodes and passed round this washer. Thus movement of the lever caused movement of the electrodes. The magnitude of the electrode movement was dependent on the point of attachment of the thread. The electrode is shown in Fig.20.

Fig.20. The electrodes used to record the effect of the application of tension "steps" on the sensory discharge.

Both the perspex levers were attached to brass rods which were fixed to a rack and pinion system such that their distance apart was alterable. The electrodes were also fixed to a rack and pinion arrangement so that they were movable in any direction.

Recording.

a) 'Steps'.

The effects of the application of tension on the discharge from the spindle were recorded by photographing the action potentials displayed on the screen of the cathode ray oscilloscope. The recording arrangements were similar to those employed in the extension experiments.

These experiments were not carried out on the screened cage but on an earthed metal table.

b) Length/Tension Relationship.

The outputs from the photo-electric and the glycerine transducers were fed into two channels of the 3-channel pen recorder (Fig.21) and recorded graphically.

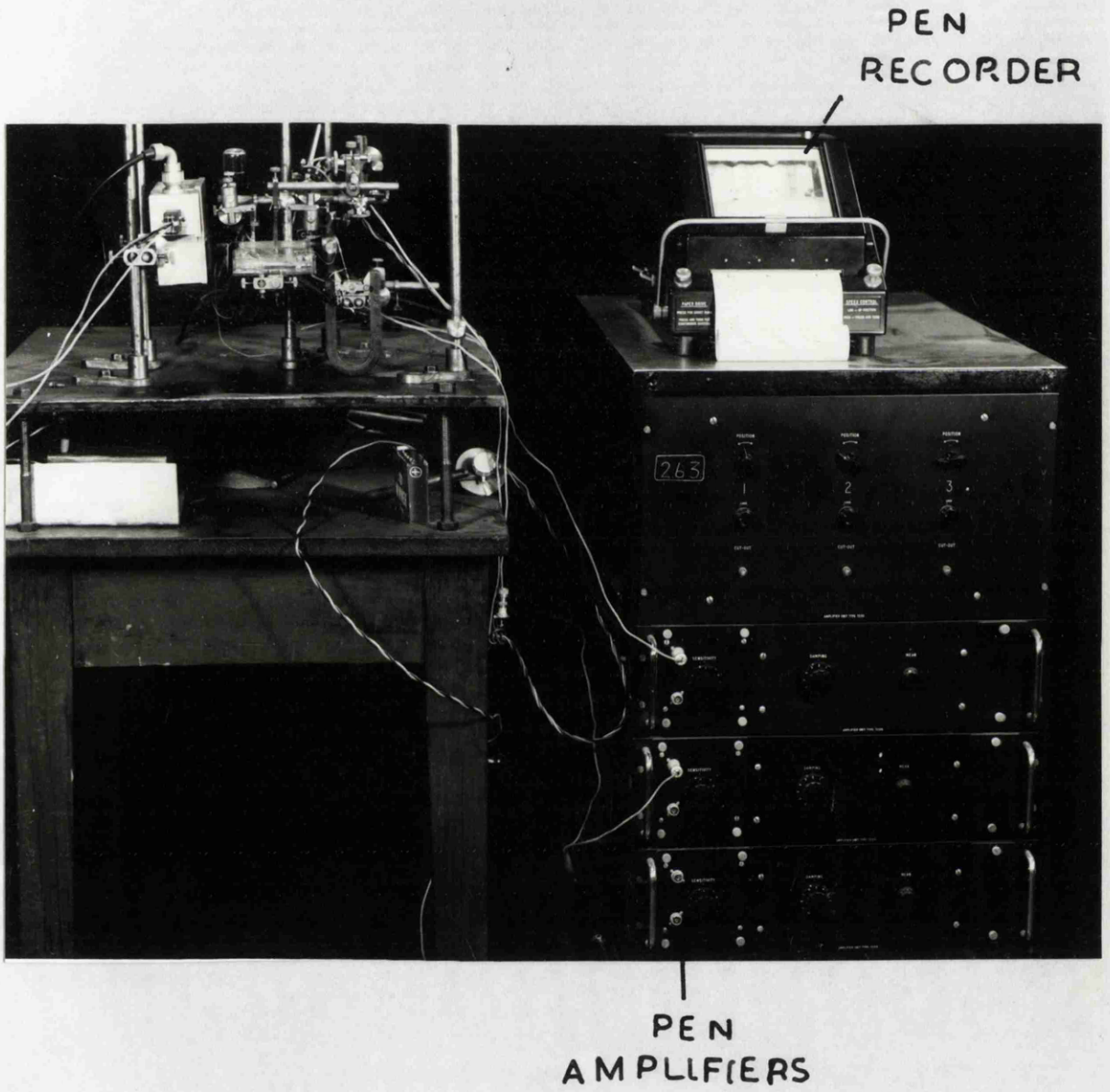


Fig.21. Photograph of the 3-channel pen-recorder with the pen amplifiers underneath. The outputs from the glycerine and photo-electric transducers were fed into amplifiers 1 and 2 and thus length and tension changes were recorded by pens 1 and 2 respectively.

c). The transfer function.

The Transfer Function.

The transfer function, derived by Roberts, Boyd & Cairnie, relates the impulse frequency, in the afferent nerve from the knee-joint proprioceptor, to the deformation at the receptor. The form of this function is shown below and the expressions derived by means of which the responses to the 'constant velocity steps' and to sinusoidal movement can be calculated.

Theory

A displacement S leads to a frequency Ψ (S) which is independent of velocity.

A velocity v leads to a frequency Φ (v) which is remembered

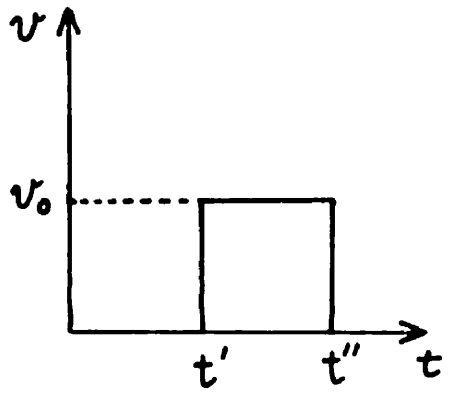
$$\delta\Phi(v_t) = \phi(v_t)\, e^{-\frac{t_1 - t}{\tau}}\, \delta t$$

where $\phi(v_t)\,\delta t$ is the component of the total frequency which is due to velocity v during the interval t to t + δ t, and τ is the time constant of decay of the memory

$$\therefore \text{ Total contribution to frequency at time } t_1 \text{ due to this component} = \int_{-\infty}^{t_1} \phi(v_t)\, e^{-\frac{t_1 - t}{\tau}}\, dt$$

(Note - For convenience, $\phi(v_t)$ is referred to below as ϕ).

Application to constant velocity steps



Suppose movement with velocity v_0 lasts from $t = t'$ till $t = t''$

Velocity component at $t_1 = \int_{-\infty}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$

$$= \int_{-\infty}^{t'} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt + \int_{t'}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$$

Velocity component at $t' = \int_{-\infty}^{t'} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt = 0$

$\therefore$ Velocity component at $t_1 = \int_{t'}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$

a) $t' < t_1 < t''$

Velocity component at $t_1 = \int_{t'}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$

Let $\phi = kv$, then if k is constant, ϕ is constant.

$\therefore$ Velocity component at $t_1 = \phi\tau \left(e^{-\frac{t_1 - t}{\tau}} \right)_{t'}^{t_1}$

$$= \phi\tau \left[1 - e^{-\frac{t_1 - t'}{\tau}} \right]$$

b) $t_1 > t''$

Velocity component at $t_1 = \int_{t'}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$

$$= \int_{t'}^{t''} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt + \int_{t''}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$$

$\phi = kv$. When $t_1 > t''$, $v = 0$

$$\therefore \phi = 0$$

$$\therefore \int_{t'}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt = 0$$

$\therefore$ Velocity component at $t_1 = \int_{t'}^{t''} \phi\, e^{-\frac{t_1 - t}{\tau}}\, dt$

$$= \phi\tau \left[e^{-\frac{t_1 - t}{\tau}} \right]_{t'}^{t''}$$

$$= \phi\tau \left[e^{-\frac{t_1 - t''}{\tau}} - e^{-\frac{t_1 - t'}{\tau}} \right]$$

Application to sinusoidal movement

Let $v = a \sin \omega t$ where $a = \omega r$

Let $\phi(v) = kv$ (found to be approximately true)

$= ka \sin \omega t$

Frequency, at time t_1 $= \int_{-\infty}^{t_1} \phi(v_t)\, e^{-\frac{t_1 - t}{\tau}} dt$

due to velocity

$$= \int_{-\infty}^{0} \phi\, e^{-\frac{t_1 - t}{\tau}} dt + \int_{0}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}} dt$$

$$\int_{0}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}} dt = ak\, e^{-\frac{t_1}{\tau}} \int_{0}^{t_1} e^{\frac{t}{\tau}} \sin \omega t\, dt$$

Now $\int_{0}^{t_1} e^{at} \sin bt\, dt = \left[e^{at} \frac{(a \sin bt - b \cos bt)}{a^2 + b^2} \right]_0^{t_1}$

$\therefore$ Substituting $a = \frac{I}{\tau}$ and $b = \omega$

We have $\int_{0}^{t} \phi\, e^{-\frac{t_1 - t}{\tau}} dt = \frac{ak\, e^{-\frac{t_1}{\tau}}}{\omega^2 + \frac{1}{\tau^2}} \left[e^{\frac{\tau}{\tau}} \left(\frac{1}{\tau} \sin \omega t - \omega \cos \omega t \right) \right]_0^{t_1}$

Put $\frac{ak}{\omega^2 + \frac{1}{\tau^2}} = C$

$$\therefore \int_{0}^{t_1} \phi\, e^{-\frac{t_1 - t}{\tau}} dt = C\, e^{-\frac{t_1}{\tau}} \left[e^{\frac{t_1}{\tau}} \left(\frac{1}{\tau} \sin \omega t_1 - \omega \cos \omega t_1 \right) + \omega \right]$$

$$= C \left[\frac{1}{\tau} \sin \omega t_1 - \omega \cos \omega t_1 + \omega e^{-\frac{t_1}{\tau}} \right]$$

Similarly $\int_{-2n\frac{\pi}{\omega}}^{0} \phi\, e^{-\frac{t_1-\tau}{\tau}} dt = C\, e^{-\frac{t_1}{\tau}}\left[e^{\frac{t}{\tau}}\left(\frac{1}{\tau}\sin\omega t - \omega\cos\omega t\right)\right]_{-2n\frac{\pi}{\omega}}^{0}$

$= C\, e^{-\frac{t_1}{\tau}}\left[-\omega - e^{-\frac{2n\pi}{\omega\tau}}(-\omega)\right]$

Let $n \to \infty$, then $e^{-\frac{2n\pi}{\omega\tau}} \to e^{-\infty} = 0$

$\therefore \int_{-\infty}^{0} \phi\, e^{-\frac{t_1-\tau}{\tau}} dt = -C\, e^{-\frac{t_1}{\tau}}\omega$

$\therefore$ Frequency, at time t_1 due to velocity $= \int_{-\infty}^{t_1} \phi\, e^{-\frac{t_1-\tau}{\tau}} dt$

$= \int_{-\infty}^{0} \phi\, e^{-\frac{t_1-\tau}{\tau}} dt + \int_{0}^{t_1} \phi\, e^{-\frac{t_1-\tau}{\tau}} dt$

$= -C\, e^{-\frac{t_1}{\tau}}\omega + C\left(\frac{1}{\tau}\sin\omega t_1 - \omega\cos\omega t_1\right) + C\, e^{-\frac{t_1}{\tau}}\omega$

$= C\left(\frac{1}{\tau}\sin\omega t_1 - \omega\cos\omega t_1\right)$

Thus it can be seen that the application of sinusoidally varying stimuli eliminates the effect of previous events on a process which shows expontial adaptation.

PART 2.

ANALYSIS OF THE RESPONSE TO EXTENSION.

Analysis.

The responses to constant velocity steps and to sinusoidal movements of the muscle were recorded on film. The intervals between impulses were measured and the course of the discharge frequency plotted on a graph. The response curves were then analysed in a similar manner to the analysis of the knee joint response. This analysis is described in detail below.

It was hoped to see whether the same transfer function would be able to predict the responses of both sense organs (i.e. cat knee joint proprioceptor and frog neuromuscular spindle) to certain types of stimulation.

Response to Constant Velocity Steps.

The effect of stretch on the discharge frequency of the spindle was recorded on film only on those occasions when a 'single unit' discharge was obtained. Film recordings were made of the responses of nine different units. In only four of these were the responses to stretch analysed. Analysis of the response to stretch is quite a lengthy procedure, so that those units which had a low discharge frequency, or in which the movement was not quite linear, were not analysed. The steps applied to each unit are shown in Table 1.

The average length of this muscle is 15 mm. and the

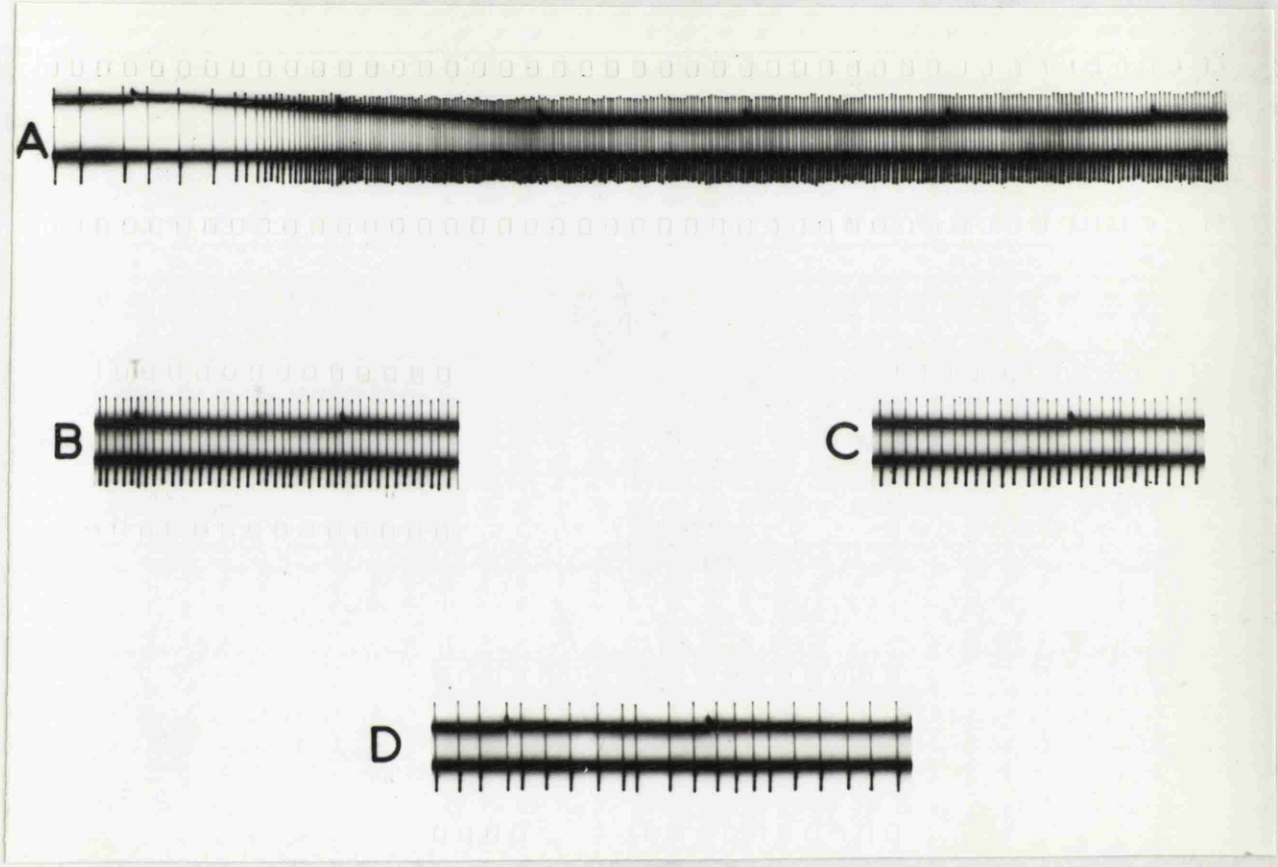


Fig.22. Oscillograph records of the effect on the sensory discharge of the application of a constant velocity step (Unit 9 (4)). The upper line signals the movement (11.6 mm. extension) and also carries the time-marker ($\frac{1}{2}$ sec. pulses).

A. Application of the step.

B. Discharge recorded 10 sec after step.

C. Discharge recorded 30 sec. after step.

D. Discharge recorded 2 min. after step.

Table 1.

The number, velocity and extent of the steps applied to each unit.

A. Units in which the response to every step was measured and graphed.

Unit	No.	Range (Scale)	Vel. mm./sec.	No. of Steps	Response Analysed
5	B	3.1-3.7	2	1	-
	C	"	2.4	2	+
	D	"	1.7	3	+
	E	"	-	3	Measured not smooth
	I	3.1-3.4	0.9	3	+
	J	"	2.2	2	+
	K	"	2	2	-
7	C	2.0-2.5	0.28	3	+
	D	"	0.55	3	+
	E	"	2.0	3	+
	F	"	1.7	3	+
	G	"	0.55	3	+
	H	"	0.33	3	+
8	3	2.9-3.4	0.25	2	+
	5	"	0.5	4	+
	7	"	1.7	3	+
	10	3.4-3.9	1.7	3	-
	12	"	0.5	3	-
	14	"	0.25	3	-
9	1	3.3-3.7	0.27	2	+
	2	"	0.51	2	+
	3	"	0.7	2	+
	4	"	1.0	2	+
	5	"	1.3	2	+
	6	"	1.8	2	+

Response to 25 steps measured and plotted.
Response to 19 steps analysed.

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Response to 57 steps recorded
Response to 48 steps measured
Response to 19 steps analysed.

Table 1 (Contd.)

B. Units in which the recorded responses were not all measured.

Unit	No.	Range (Scale)	Vel. mm./sec.	No. of Steps	Response Plotted	
9	12	3.3-4.0	0.29	1	+	
	13	"	0.44	1	+	
	14	"	0.58	1	+	
	15	"	0.93	1	+	Records
	16	"	1.4	1	+	too
	17	"	2.1	1	+	short
	18	3.3-4.3	2.0	1	+	for
	19	"	1.5	1	+	analysis
	20	"	1.28	1	+	
	21	"	0.74	1	+	
	23	"	0.3	1	+	
	27	3.7-4.3	0.25	1	+	
	28	"	0.52	1	+	
	29	"	0.63	1	+	
	30	"	0.82	1	+	
	31	"	1.04	1	+	
	32	"	1.85	1	+	
1		Film speed unsuitable			-	
2	A				-	
	B				-	
	C				-	
3	B	Freq. very low			-	
	C					
	D					
4	B	Freq. very low			-	
	C					
	D					
6	C	4.0-4.5	0.2	1	+	
	D	"	0.40	3	+	
	E	"	0.66	2	+	
	G	4.5-5.0	0.2	2	+	
	H	"	0.3	2	+	
	N	4.0-5.0	0.2	4	+	

Response to 32 steps recorded
Response to 23 steps measured and plotted.

maximum extension was 2.4 mm. The time taken for the steps varied with velocity, with a maximum time of 4 sec.

The response to a constant velocity step was recorded continuously for about 30 sec. after the application of stretch and thereafter for 5 sec. periods, $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 minutes later. The muscle was then restored to its original position and a 3 minute interval allowed to elapse before the next stretch. An attempt was made, at each velocity of stretch, to repeat three steps over the same range.

It was found that a 3-minute interval between pulls gave consistent responses (as shown in Fig.23). If there was a longer interval (e.g. when the camera film had to be changed between pulls) it was occasionally found that the response to the second stretch was somewhat greater than that previously obtained. This effect is shown in Fig.24.

From the protocol there are 18 occasions (Table 2) when there was a longer-than-normal interval between pulls (though there is an impression that in some of the later experiments an unrecorded pull was made to offset the effect of the longer pause). In 7 of these occasions the subsequent response was higher than normal. If this effect is genuine it may mean that 3 min. was too short a period to allow for the return to full excitability of the spindle.

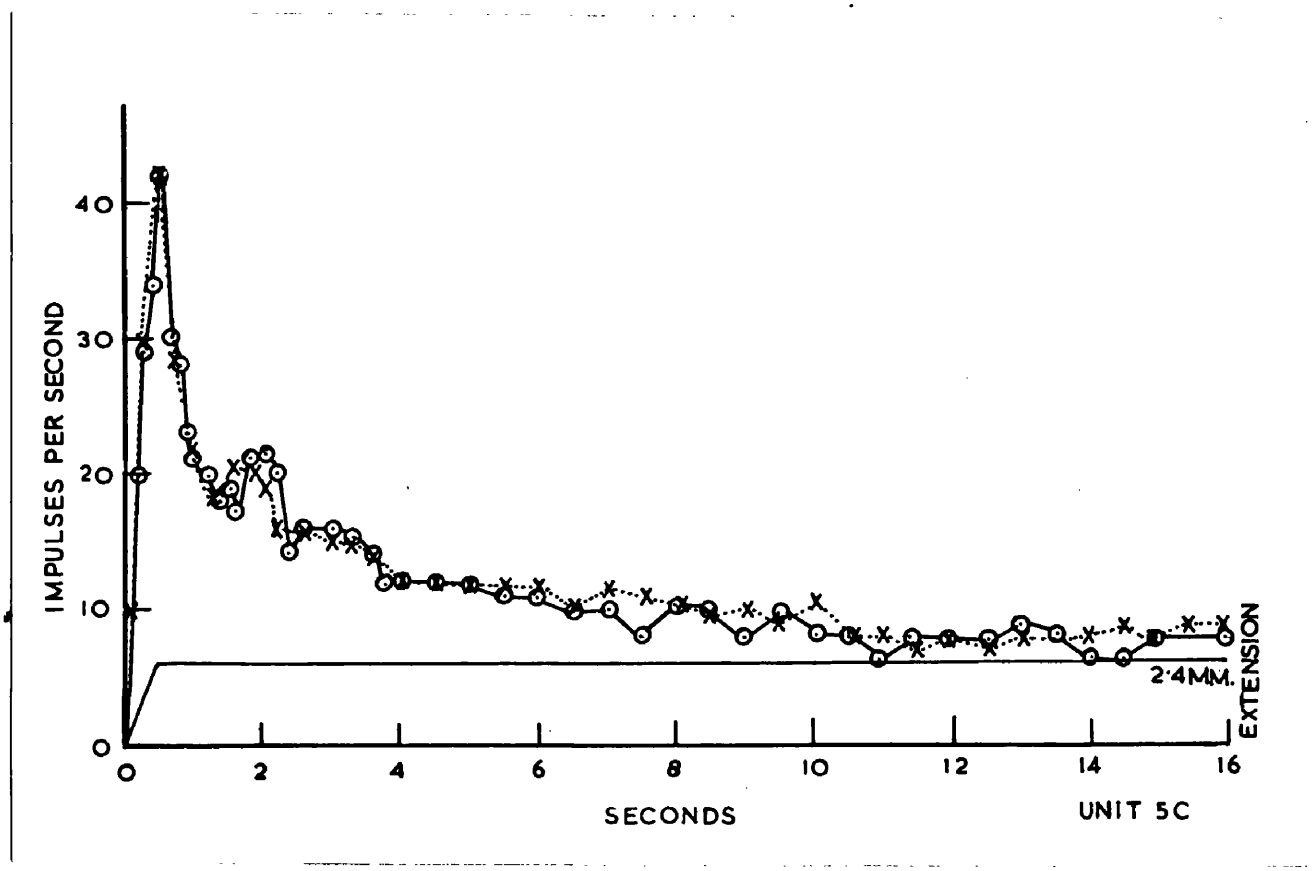

Fig.23. Graphs of the responses to two successive steps at the same velocity, over the same range, showing the repeatability of the response

⊙——⊙ 1st step.

X·····X 2nd step.

The muscle was kept in the stretched position for 2 min. It was then restored to its original position and 3 min. allowed to elapse before repetition of the step.

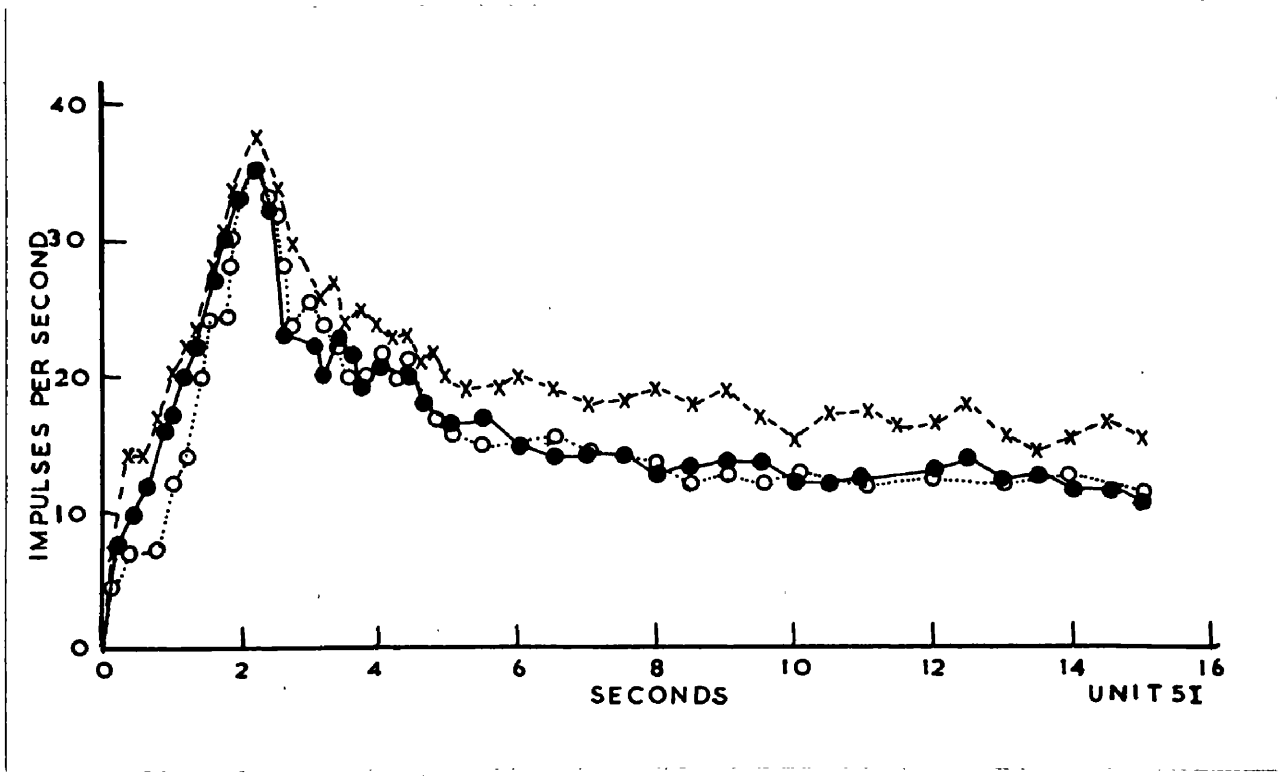

Fig.24. Graphs showing the effect on the response of allowing a longer interval than 3 min. between steps.

3 steps at the same velocity over the same range.

⊙ ·····⊙ 1st step.

× ------ × 2nd step (after 10 min. interval).

● ——— ● 3rd step (3 min. after 2nd step).

Table 2.

Occasions when the interval between repeated steps (at the same vel. over the same range) was greater than normal.

Unit	No. of longer-than-3 min. pauses	Effect on the response
5	5	3 rises
7	5	1 rise
8	4	-
9	4	3 rises
	18 pauses	7 rises

On 18 occasions the interval between pulls was longer than the normal 3 min.

On 11 occasions the subsequent response was unchanged, while on 7 occasions there was a rise in the response.

Displacement Component, $\Psi(s)$

In the theory of the transfer function it was postulated that one portion of the response was due to the position, or deformation, of the receptor. This is the displacement component, $\Psi(s)$.

From Fig.23, it can be seen that at about 15 sec. after the end of constant velocity step the discharge frequency from the spindle has settled down to a steady value. Two minutes after the movement the frequency is still at this level.

If the graphs of the responses to steps at different velocities, over the same range, are superimposed, as in Fig.25, it can be seen that the discharges all adapt to the same steady value. Therefore this frequency must be independent of velocity. The frequency of the response after two minutes in any position is taken to be due solely to its position, i.e. it is the displacement component Ψ .

It was assumed that this value Ψ was attained and kept whenever the final position was reached and that, during movement, this component increased linearly with position.

On the occasions when the discharge frequency was measured after 3 min. in various positions and these values plotted against the extension of the muscle this linear relationship appeared to be approximately true. These position/frequency curves are shown in Fig.26.

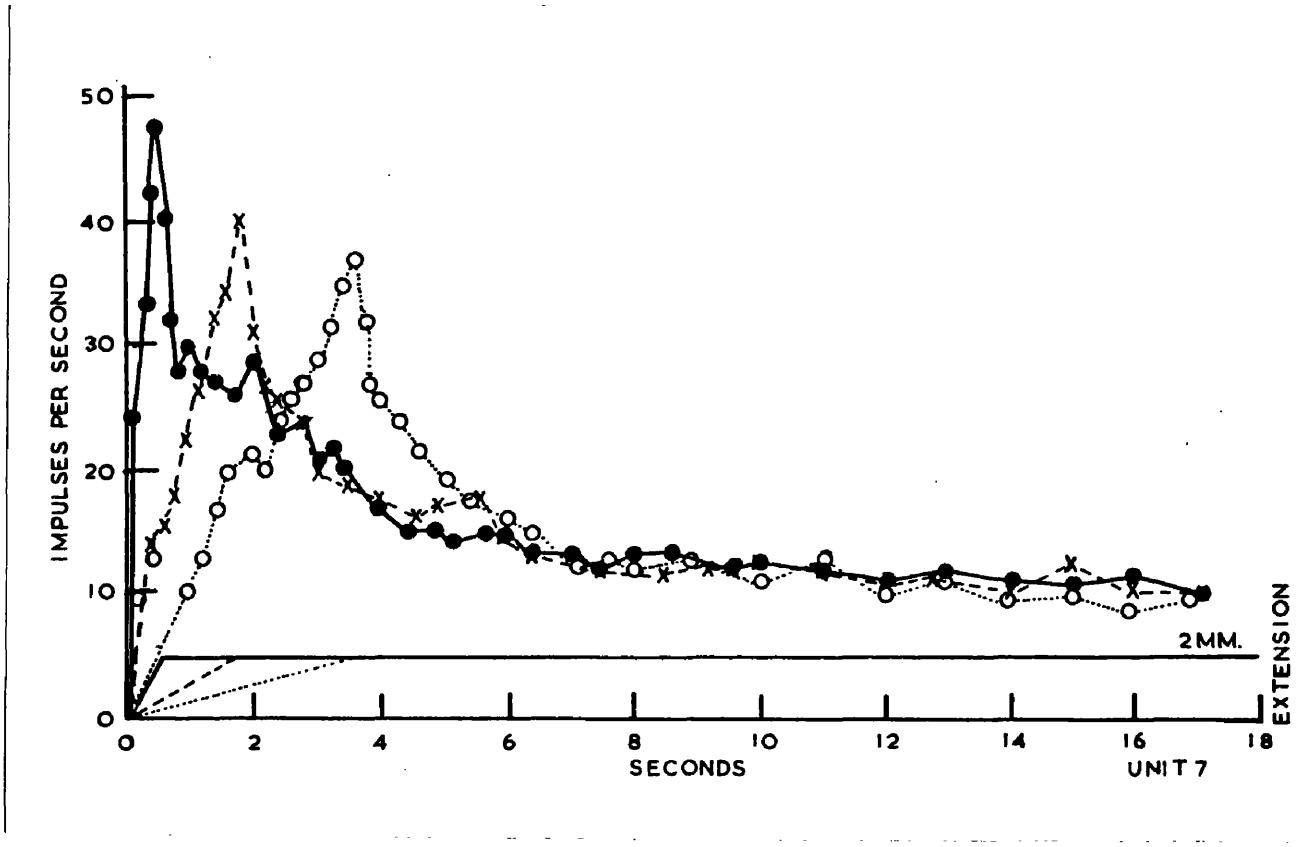

Fig.25. Graphs of the responses to steps of three different velocities over the same range.

The three responses adapt to the same final value.

● ——— ● 4 mm./sec.

× ------ × 1.1 mm./sec.

⊙ ..... ⊙ 0.6 mm./sec.

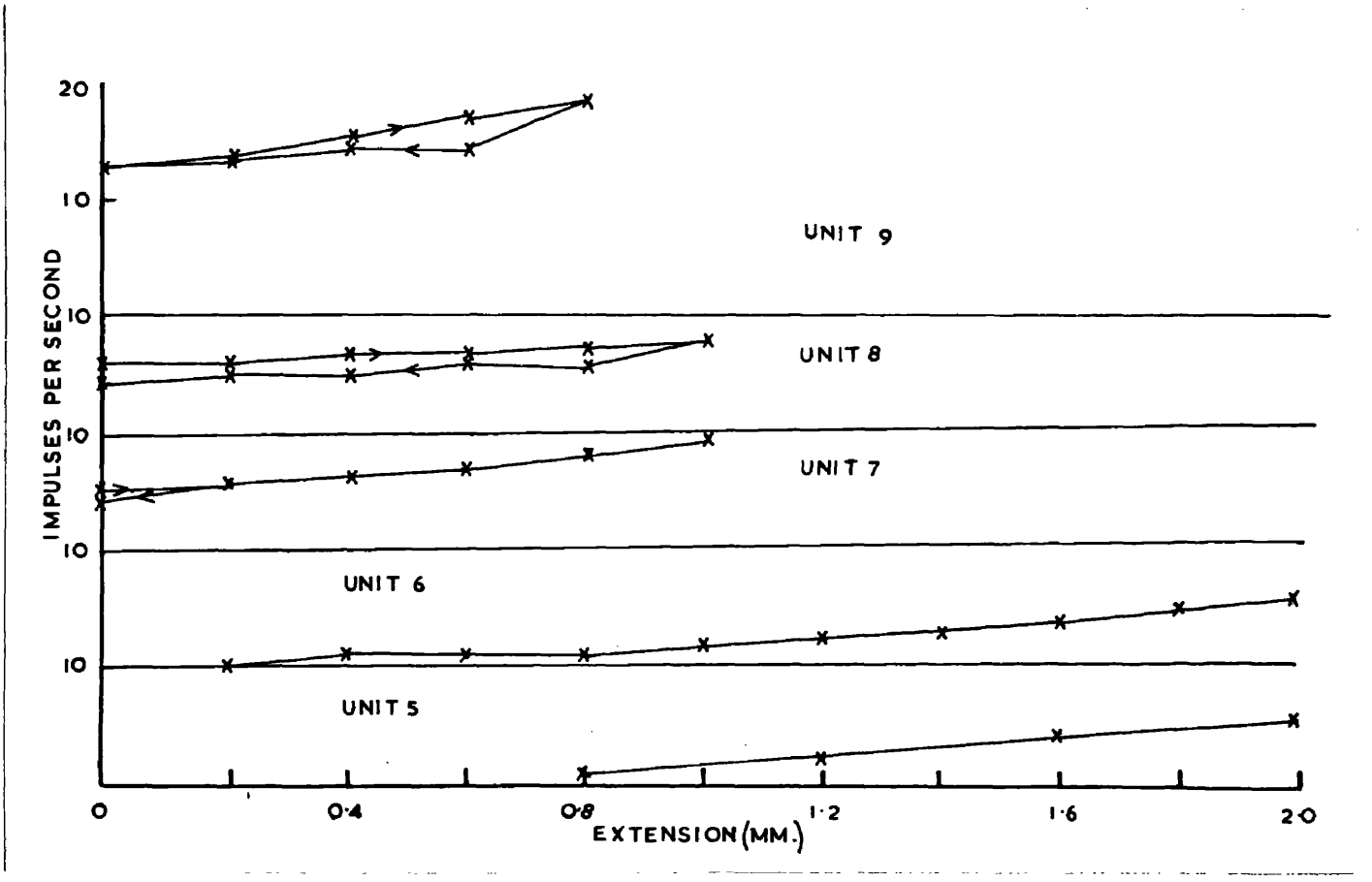

Fig.26. The relationship between extension of the muscle and discharge frequency for units 5, 6, 7, 8, 9.

Each point represents the frequency of the discharge recorded 3 minutes after the corresponding position had been reached.

This relationship between position and adapted discharge frequency is approximately linear.

(The ordinate scale starts afresh from zero for every unit.)

Unit 9 differs from the other units in that, although the frequency of the responses to steps of different velocity have all adapted to almost the same value after 18 seconds, this value is higher than the value of the response after 2 minutes (Fig.27).

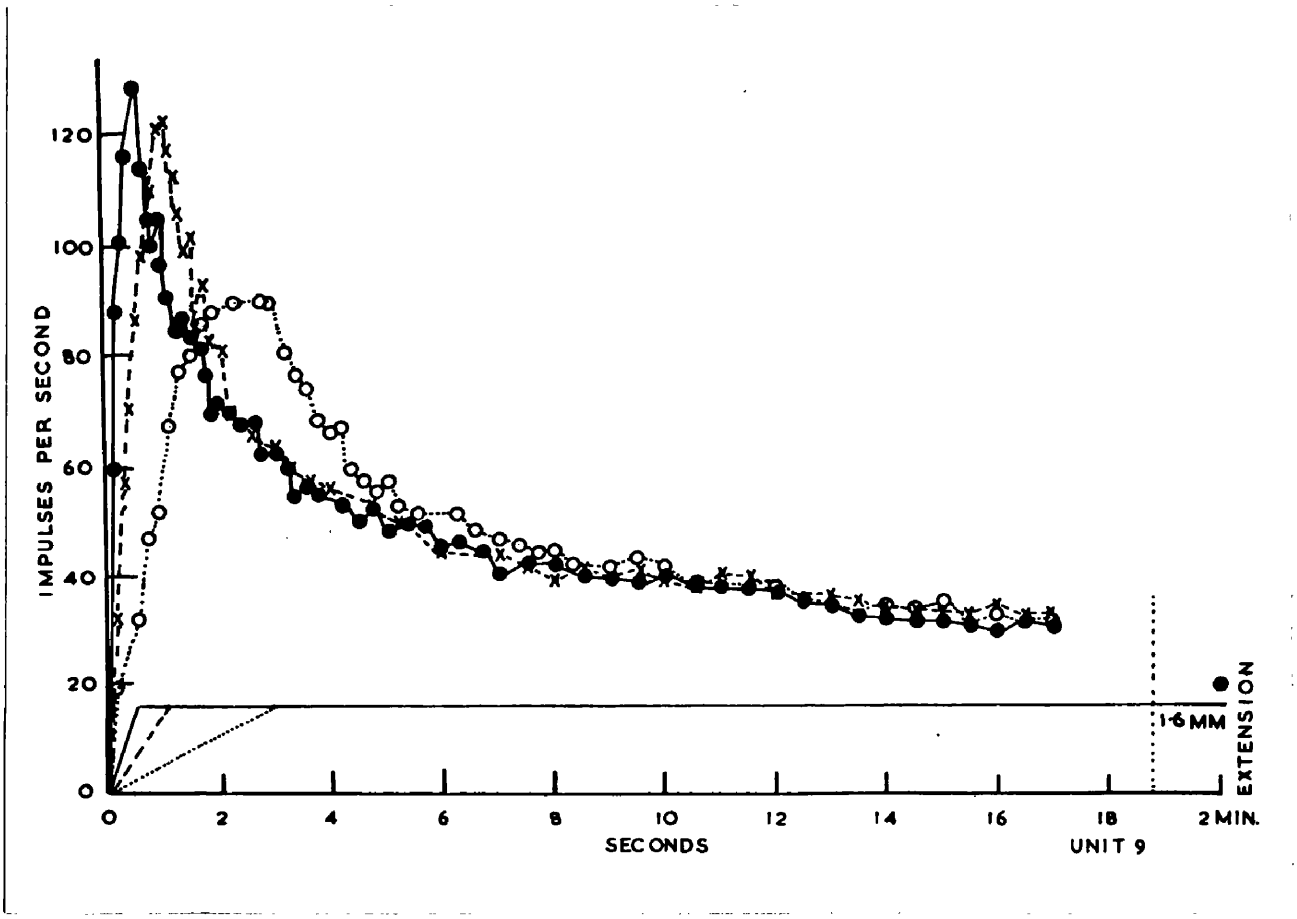

Fig.27. Graphs of the responses to steps of three different velocities over the same range.

● ——— ● 2.7 mm./sec.

× ------ × 1.1 mm./sec.

⊙ ..... ⊙ 0.5 mm./sec.

17 sec. after the start of the movement, responses have all adapted to 32 imp./sec.

2 min. after the start of the movement, responses have all adapted to 20 imp./sec.

Velocity Component $\Phi(v)$

It was postulated that the total response F equalled the sum of the displacement component $\Psi(s)$ and the velocity component $\Phi(v)$. Thus when the displacement component was deducted from the total response curve F, as shown in Fig.28, the curve which remained was assumed to be composed solely of components due to the velocity of the movement.

When the muscle was stretched by steps of constant velocity, the response frequency/time curves obtained were similar in form to those which were obtained from the cat knee-joint proprioceptor (Fig.29) in response to similar movements. It was found that, for the knee-joint proprioceptor, the assumption that there were three velocity components, Φ_1, and Φ_3 positive, and Φ_2 negative, gave quite a good fit of the experimental curve. Therefore it seemed reasonable to attempt to fit three velocity components to the spindle response.

Φ_3 The form of the response curve, initial rapid fall and long tail, made it seem likely that there was a component with a short memory and one with a long memory. In the latter part of the curve the impulse frequency will be entirely due to this long memory component, say Φ_3. From examination of this part of the curve, which decayed approximately exponentially, a value was chosen for the time constant of decay τ_3.

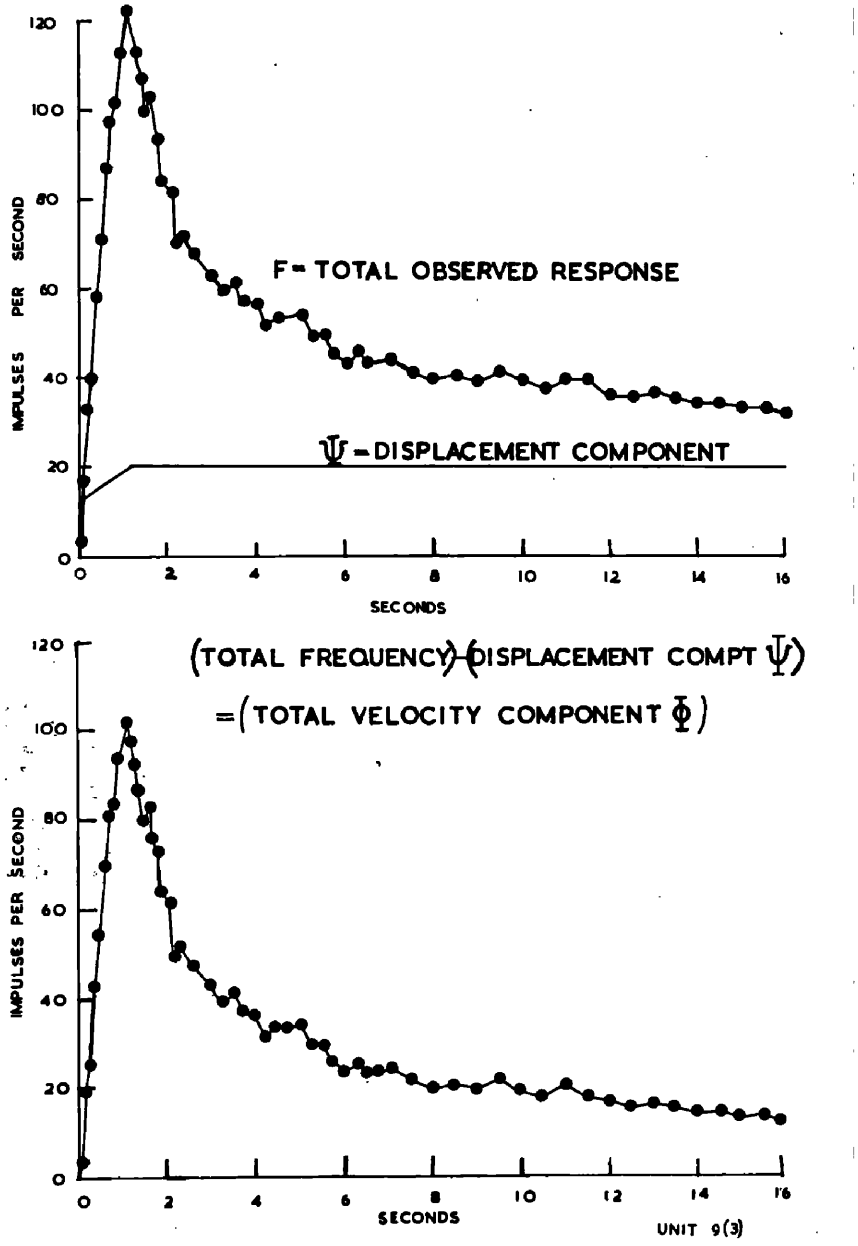

Fig.28. A. The total observed response, F, and the displacement component, Ψ.

B. Total observed response less the displacement component, giving the part of the response postulated to be due solely to the velocity of the movement.

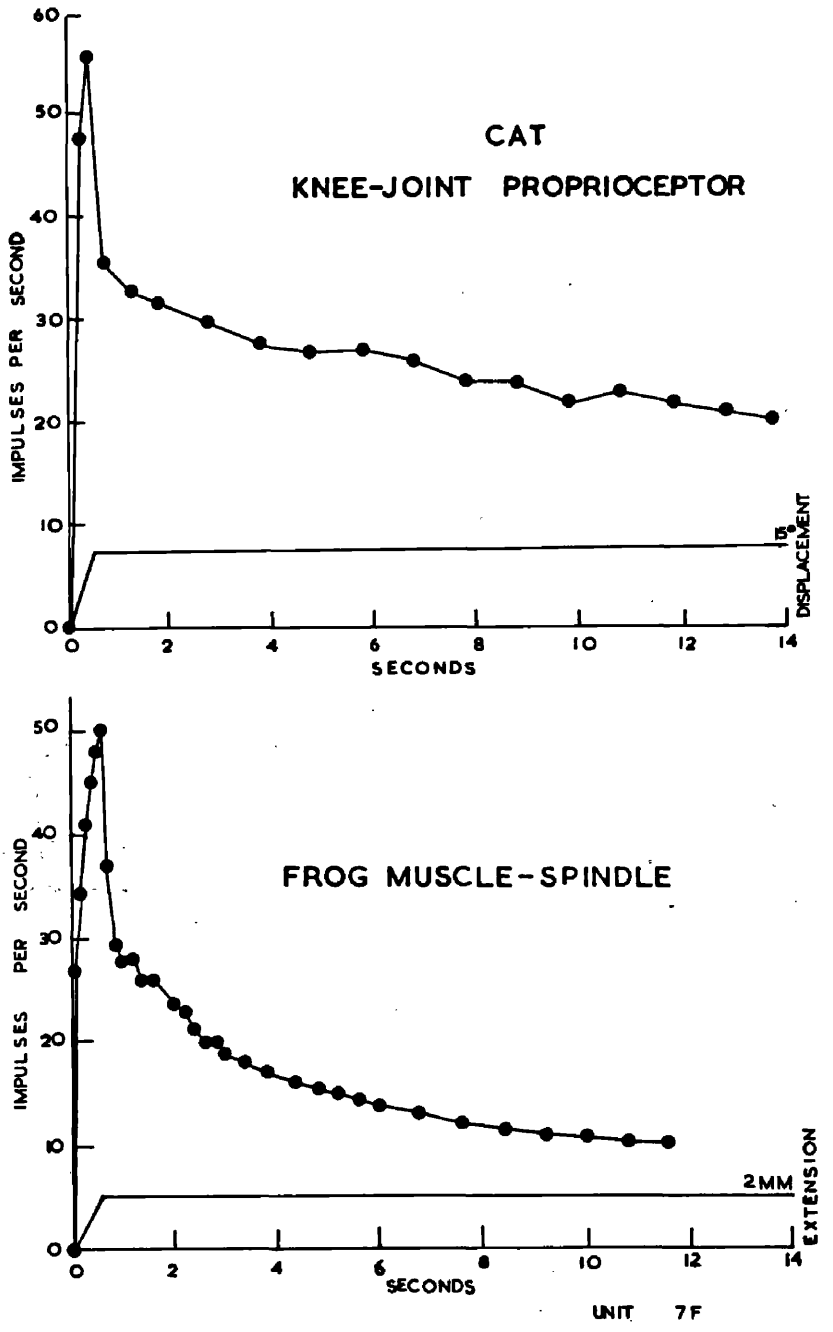

Fig.29. Graphs of the responses of the cat knee-joint proprioceptor and of the frog muscle spindle to similar constant velocity steps.

A representative point X, at a time t_1, was chosen arbitrarily on this portion of the curve. The frequency at t_1 was assumed to be due solely to the component Φ_3 From the formula for the response after the end of the movement,

$$\text{Frequency at time } t_1 = \phi_3 \tau_3 \left(e^{-\frac{t'' - \tau_1}{\tau_3}} - e^{-\frac{t''}{\tau_3}} \right)$$

where t'' = duration of the movement.

ϕ_3 is the only unknown in the above equation and it can thus be determined.

Using these values of ϕ_3 and τ_3, the velocity component Φ_3 was calculated and plotted on the same graph. A reasonable fit of the tail of the experimental curve as shown in Fig.30, was taken as an indication that the values chosen for ϕ_3 and τ_3 were satisfactory.

Φ_1 and Φ_2

The velocity component Φ_3 was then deducted from the total velocity component. The curve which remained (Fig.31) was examined to see if it could be fitted by a single exponential curve. In all but two cases, this was impossible. It was therefore thought that it might be able to be fitted by two exponential curves, one positive and one negative as in the analysis of the knee-joint proprioceptor response.

To do this an attempt was made to find a curve C such that $\Phi_3 - C$ was an exponential function, and the total

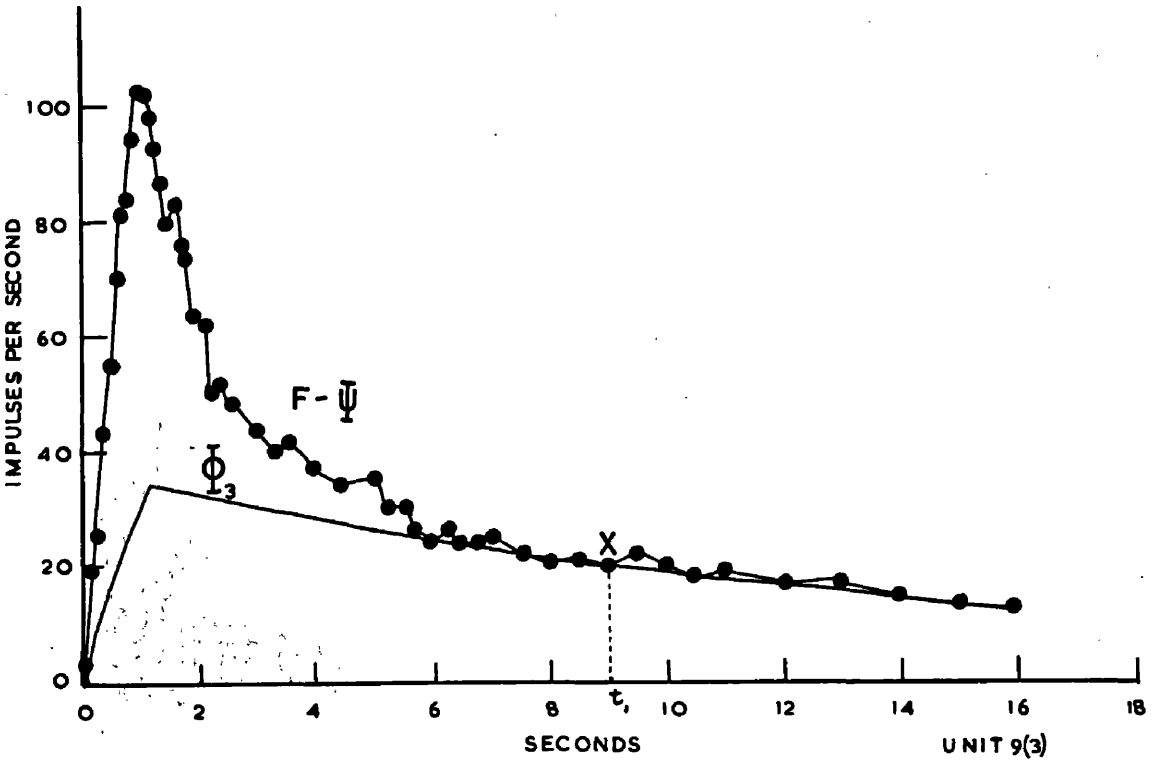

Fig.30. The velocity component Φ_3 fitted to the latter part of the total velocity response curve F - Ψ

X, at time t_1, is a point on the response curve, at which the response frequency is postulated to be due solely to the component Φ_3

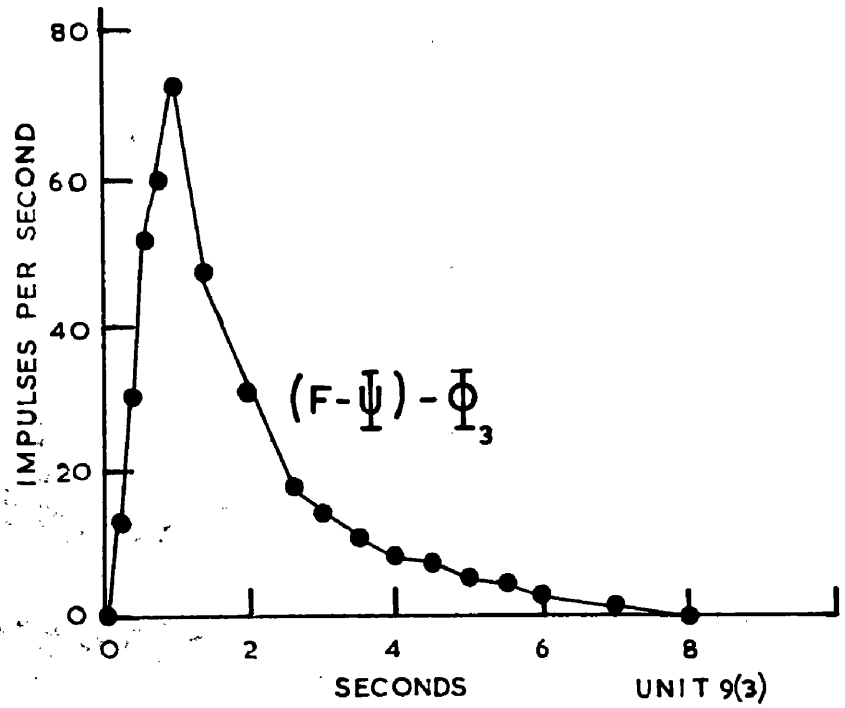

Fig.31. The part of the velocity component which remains after the deduction of the component Φ_3

velocity component $(F - \bar{\Psi})$, -C was also an exponential. Such a curve could always be found by trial and error, and it was drawn by eye as shown in Fig.32.

The two curves $\bar{\Phi}_3 - C = \bar{\Phi}_2$

and $(F - \bar{\Psi}) - C = \bar{\Phi}_1$

were plotted and their time constants of decay τ_2, and τ_1 evaluated.

From the theory, for each velocity component, the peak frequency of the response, which occurs at the end of the movement, is given by:

$$\text{Peak frequency} = \phi_i \tau_i \left(1 - e^{-\frac{t''}{\tau_i}}\right) \qquad i = 1, 2, 3.$$

t'' = duration of movement.

Since the peak frequencies and the time constants τ_1 and τ_2 were known, the values of the parameters ϕ_1 and ϕ_2 could be determined and the velocity components $\bar{\Phi}_1$ and $\bar{\Phi}_2$ evaluated. These, along with $\bar{\Phi}_3$, are shown in Fig.33, which also shows the summation of these three components.

The displacement component $\bar{\Psi}$ was then added to the total velocity component and the sum $\bar{\Psi} + \bar{\Phi}$ compared with the observed response (Fig.34). This figure also shows the sum of the displacement component and a single exponential fitted to the first part of the velocity response curve, indicating the need for more than one velocity component.

The procedure involved in the analysis of a step is illustrated below.

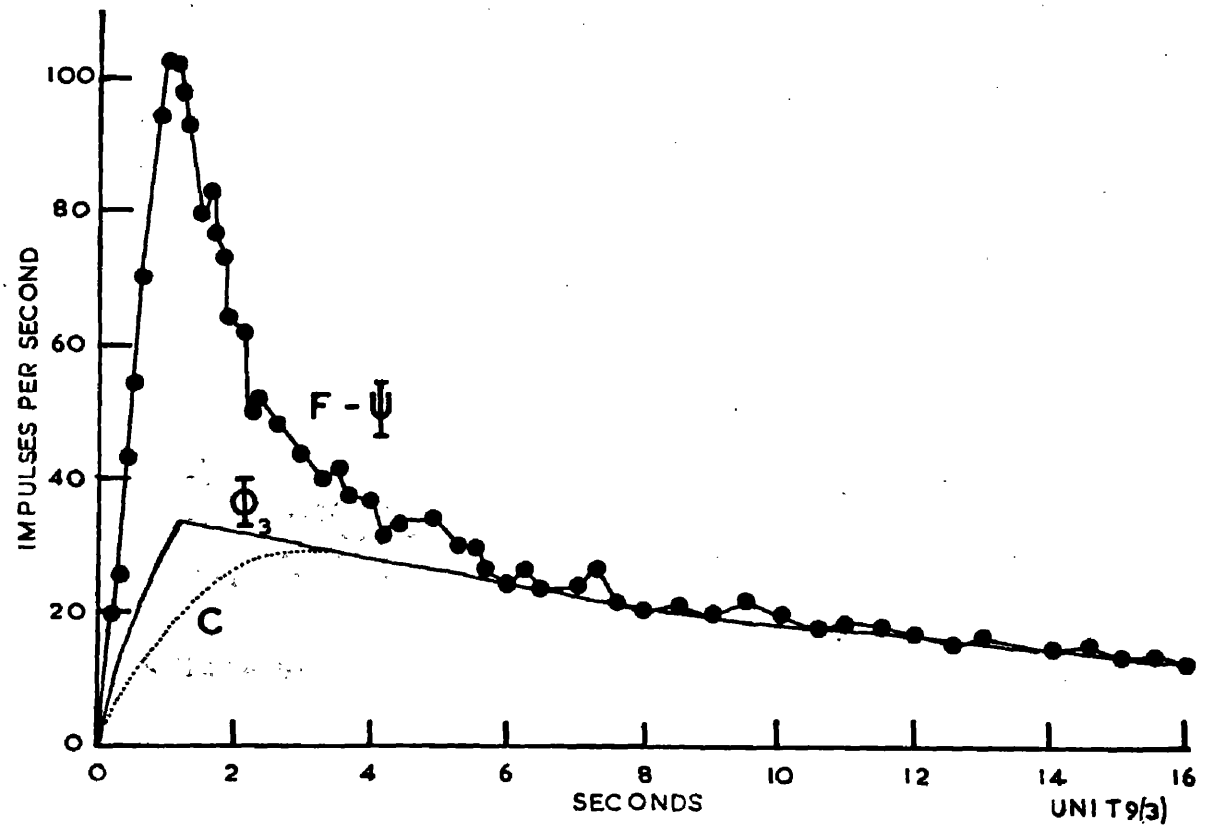

Fig.32. The curve C which was drawn by eye such that

$$(F - \Psi) - C = \text{exponential}, \Phi_1$$

$$\Phi_3 - C = \text{exponential}, \Phi_2$$

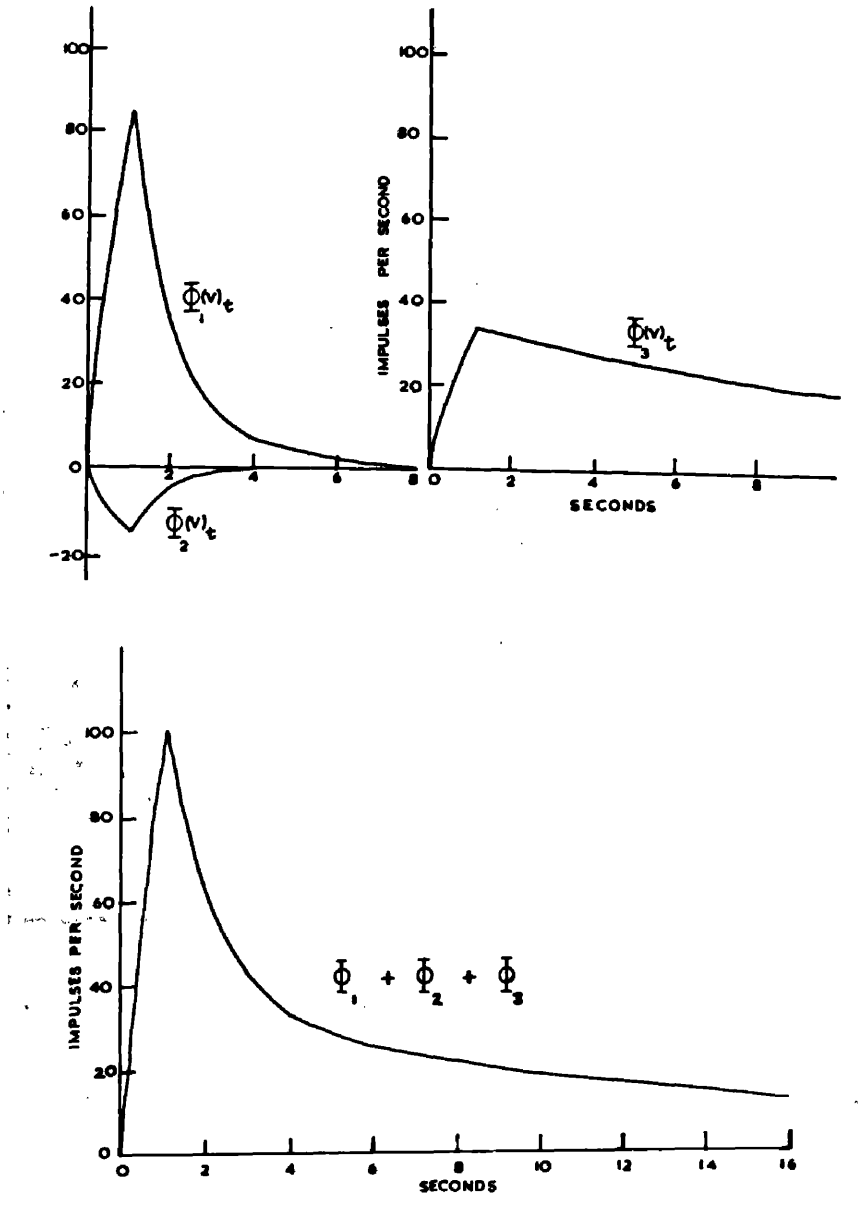

Fig.33. The velocity components of the response, Φ_1, Φ_2 and Φ_3 and their sum, the total velocity component.

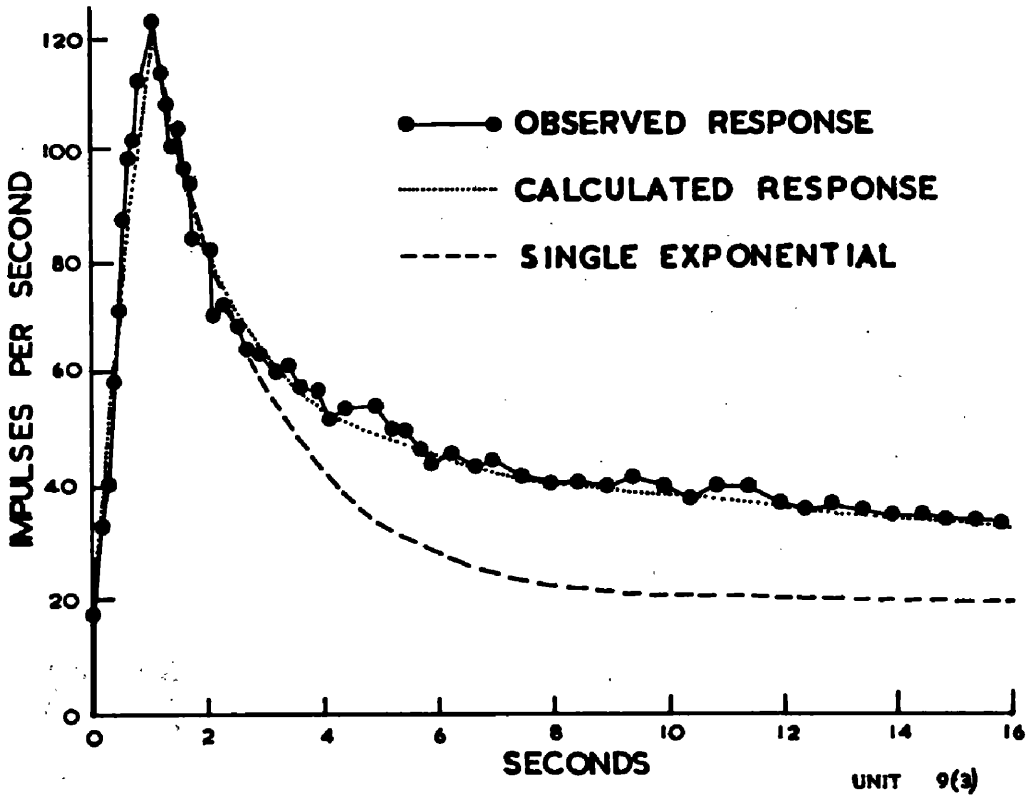

Fig.34. A comparison of the observed response (● ——— ●), the sum of the displacement and velocity components (calculated response ·····), and the sum of the displacement component and a single exponential (- - - - - - - -) fitted to the first part of the response curve.

Analysis of a Step.

Unit 9(3).

The response to two successive steps, at the same velocity, is shown in Fig.35. The value of the response after 2 min. is shown, giving the displacement component, $\Psi(s)$

The remainder of the response is then analysed into the velocity components Φ_1, Φ_2, Φ_3

Duration of movement $t'' = 1.13$ sec.

From inspection of the tail of the response curve $\tau_3 = 15$ sec.

At the point X (Fig.36), when $t_1 = 9$, frequency = 20.

From theory, frequency at X = $\phi_3 \tau_3 \left(e^{-\frac{t_1 - t''}{\tau_3}} - e^{-\frac{t_1}{\tau_3}}\right)$

$$\therefore \quad 20 = 15\, \phi_3 \left(e^{-\frac{9-1.13}{15}} - e^{-\frac{9}{15}}\right)$$

$$\therefore \quad \phi_3 = \frac{20}{15 \times \left(e^{-\frac{9-1.13}{15}} - e^{-\frac{9}{15}}\right)} = 31.1$$

Since Φ_3 and τ_3 are now known, Φ_3 can be evaluated as shown below.

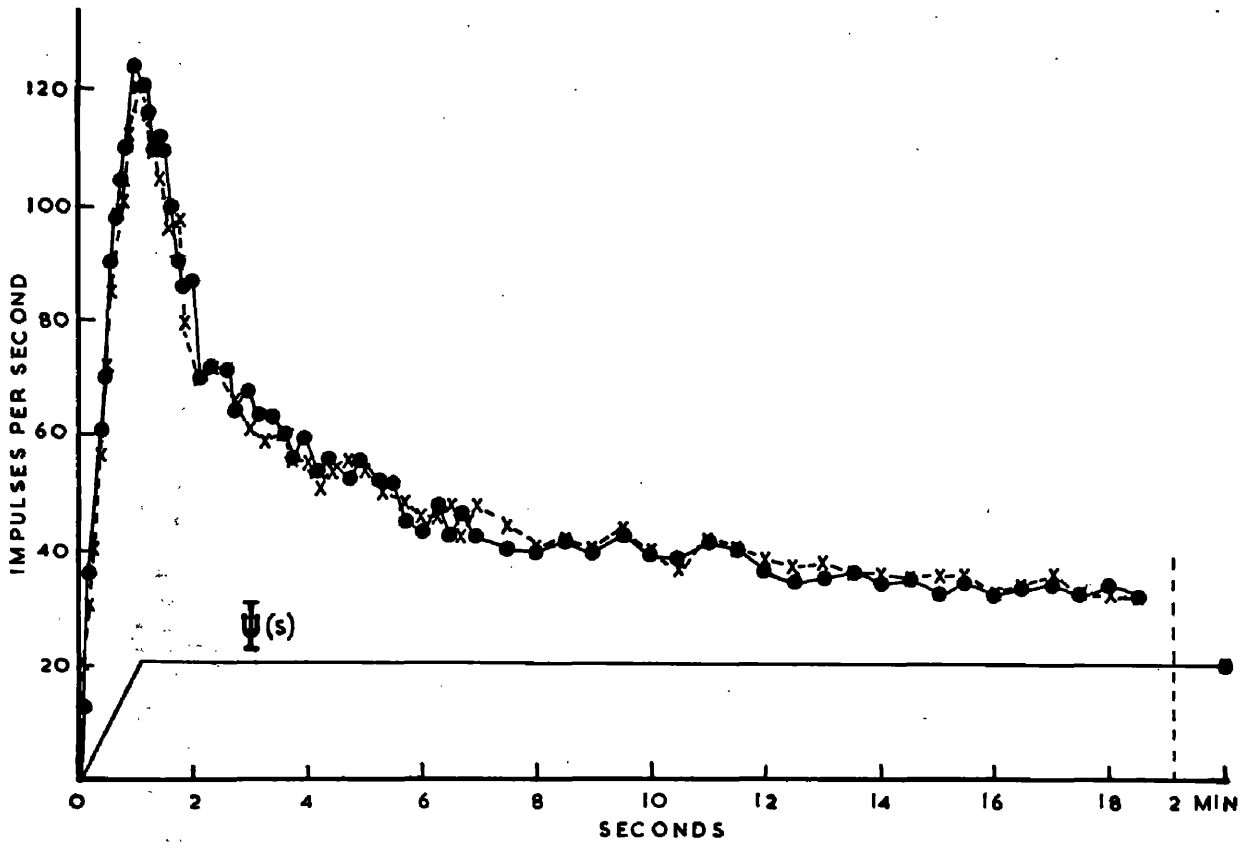

Fig.35. Graphs of the responses to two repeated steps at the same velocity, over the same range

● ——— ◆ 1st step

× ----- × 2nd step.

The value to which both responses have adapted after 2 min. is shown. This is taken to be the displacement component

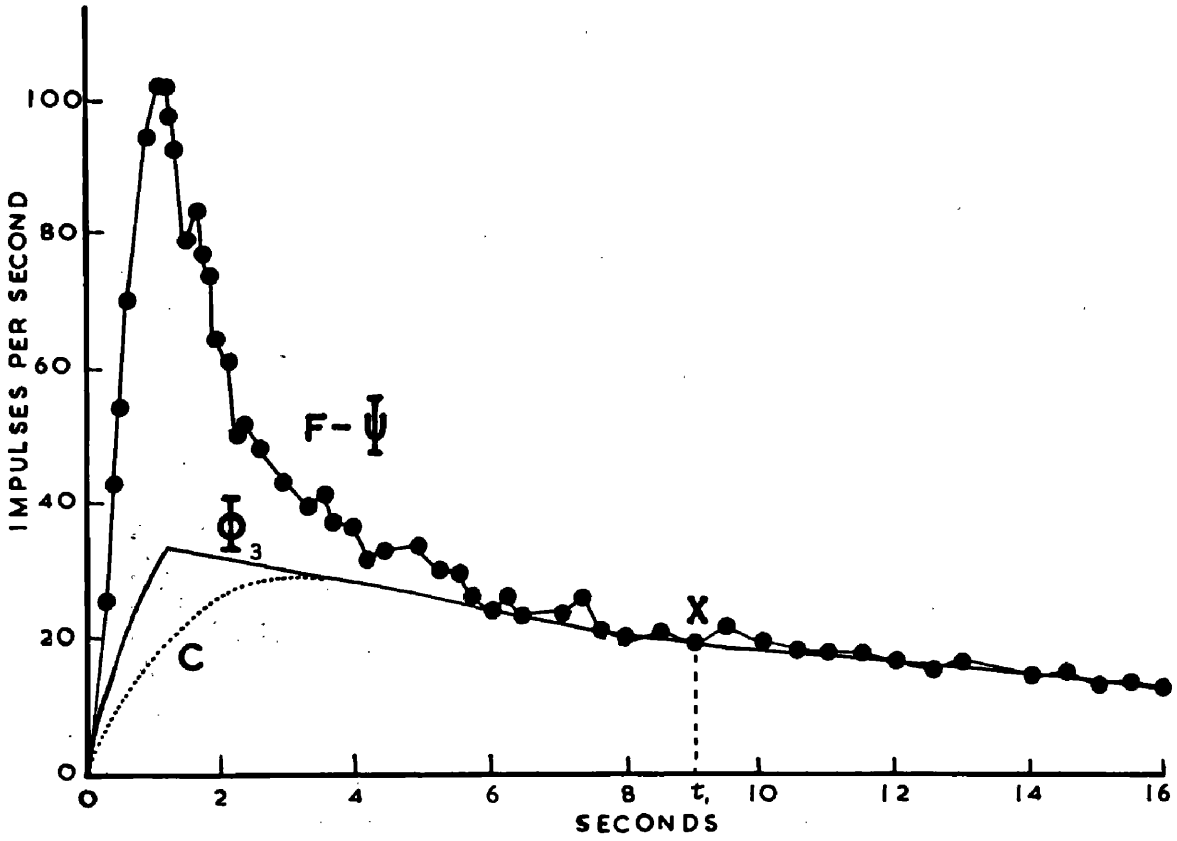

Fig.36. The component Φ_3 fitted to the tail of the part of the response due solely to velocity.

The freq. X, at time t_1, is assumed to be due solely to component Φ_3 of the response

C (·····) is drawn such that

$(F - \Psi) - C$ = exponential

and $\Phi_3 - C$ = exponential.

Evaluation of Φ_3.

t_1	$t_1-1.13$	$\frac{t_1}{15}$	$\frac{t_1-1.13}{15}$	$e-\frac{t_1}{15}$	$e-\frac{t_1-1.13}{15}$	Diff.	Φ_3
0		0				0	0
.2		.01333		.98674		.01326	6.19
.4		.02667		.97353		.02647	12.35
.6		.04		.9608		.0392	18.29
.8		.05334		.94813		.05187	24.2
1.0		.06667		.93571		.06429	29.99
1.13	0	.07533	0	.92766	1	.07234	33.75
1.5	0.37	.1	.02467	.90484	.97551	.07067	32.97
2	0.87	.13333	.058	.87534	.94382	.06848	31.95
2.5	1.37	.16667	.09134	.84651	.91274	.06623	30.9
3	1.87	.2	.12467	.81873	.88277	.06404	29.88
3.5	2.37	.23333	.158	.79181	.85383	.06202	28.93
4	2.87	.26667	.19133	.76588	.82584	.05996	27.97
4.5	3.37	.3	.22467	.74082	.79882	.058	27.06
5	3.87	.33333	.258	.71661	.77264	.05603	26.14
6	4.87	.4	.32467	.67032	.7228	.05248	24.48
7	5.87	.46667	.39134	.62708	.67622	.04914	22.93
8	6.87	.53333	.458	.58677	.63256	.04579	21.36
9	7.87	.6	.52467	.54881	.59168	.04287	20.0
10	8.87	.66667	.59133	.51445	.55367	.03922	18.3
11	9.87	.73333	.658	.48029	.5179	.03761	17.55
12	10.87	.8	.72467	.44933	.48449	.03516	16.4
13	11.87	.86667	.79133	.42033	.45327	.03294	15.37
14	12.87	.93333	.858	.39324	.42401	.03077	14.35
15	13.87	1.	.92467	.36788	.39668	.0288	13.44
16	14.87	1.06667	.99133	.34416	.37107	.02691	12.55

The curve C is then drawn by eye. The curves $(F-\Psi)-C$ and $\Phi_3 - C$ are drawn. If these decay approximately exponentially, C is considered satisfactory.

From the curve $(F-\Psi)-C$, the time constant of decay, τ_1, is determined.

$$\tau_1 = 1.1 \text{ sec.}$$

The peak frequency at end of movement $P_i = \phi_i \tau_i \left(1 - e^{-\frac{t''}{\tau_i}}\right) \quad i = 1, 2, 3$

$$\therefore P_1 = 82.5 = 1.1\ \phi_1 \left(1 - e^{-\frac{1.13}{1.1}}\right)$$

$$\therefore \phi_1 = \frac{82.5}{1.1\left(1 - e^{-\frac{1.13}{1.1}}\right)} = 116.7$$

Similarly $\tau_2 = 0.8$ sec.

$$P_2 = 16 = 0.8\ \phi_2 \left(1 - e^{-\frac{1.13}{0.8}}\right)$$

$$\therefore \phi_2 = \frac{16}{0.8\left(1 - e^{-\frac{1.13}{0.8}}\right)} = 26.4$$

Since τ_1, τ_2 and ϕ_1 and ϕ_2 are known the components Φ_1 and Φ_2 can be evaluated as shown below.

Evaluation of Φ_1 .

t_1	$t_1-1.13$	$\frac{t_1}{1.1}$	$\frac{t_1-1.13}{1.1}$	$e-\frac{t_1}{1.1}$	$e-\frac{t_1-1.13}{1.1}$	Diff.	Φ_1
0		0		1		0	0
.2		.18182		.8339		.1661	21.35
.4		.36364		.69504		.30496	39.19
.6		.54545		.5796		.4204	54.13
.8		.72727		.48323		.51677	66.41
1.0		.90909		.4029		.5971	76.74
1.13	0	1.02727	0	.35797	1	.64203	82.51
1.5	0.37	1.36364	.33636	.25573	.7145	.45877	58.97
2	0.87	1.81818	.79091	.16233	.45347	.29114	37.42
2.5	1.37	2.27273	1.24545	.10304	.2878	.18476	23.75
3	1.87	2.72727	1.7	.06539	.18268	.11729	15.07
3.5	2.37	3.18182	2.15454	.04151	.11596	.07445	9.57
4	2.87	3.63636	2.60909	.02635	.07361	.04726	6.07
4.5	3.37	4.09091	3.06364	.01672	.04672	.03	3.86
5	3.87	4.54545	3.51818	.01062	.03056	.01994	2.56
6	4.87	5.45454	4.42727	.00428	.01195	.00767	0.99
7	5.87	6.36364	5.33636	.00172	.00481	.00309	0.4
8	6.87	7.27273	6.24545	.00069	.00194	.00125	0.16

Evaluation of Φ_2.

t_1	$t_1-1.13$	$\frac{t_1}{0.8}$	$\frac{t_1-1.13}{0.8}$	$e-\frac{t_1}{0.8}$	$e-\frac{t_1-1.13}{0.8}$	Diff.	Φ_2
0		0		1		0	0
.2		.25		.7788		.2212	4.6
.4		.5		.60653		.39347	8.3
.6		.75		.4724		.5276	11.16
.8		1.0		.36788		.63212	13.37
1.0		1.25		.2865		.7135	15.09
1.13	0	1.4125	0	.24354	1	.75646	16.0
1.5	0.37	1.875	.4625	.15335	.62975	.4764	10.07
2	0.87	2.5	1.0875	.08208	.33706	.25498	5.39
2.5	1.37	3.125	1.7125	.04394	.18042	.13648	2.89
3	1.87	3.75	2.3375	.02246	.09656	.0741	1.57
3.5	2.37	4.375	2.9625	.01259	.05169	.0391	0.83
4	2.87	5	3.5875	.00674	.02767	.02093	0.44

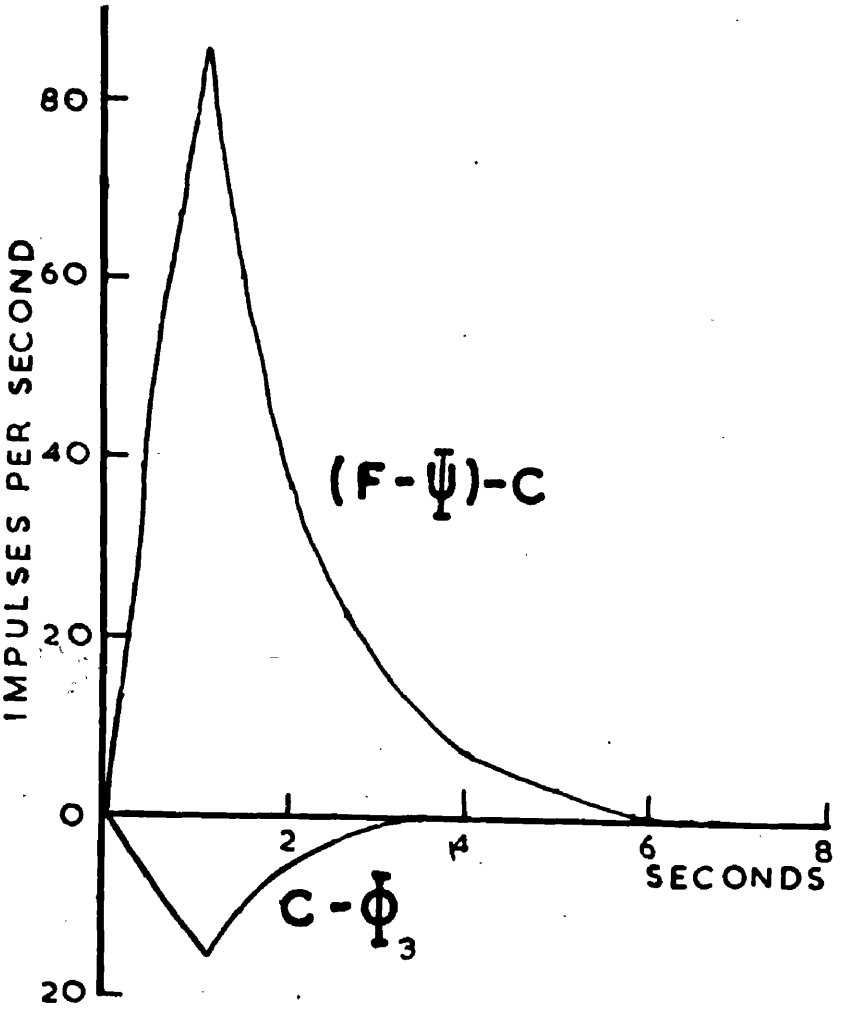

Fig.37. The curves $(F - \Psi) - C$, and $C - \Phi_3$, which are drawn out to see whether they are exponential in form.

These three velocity components were then summed giving the total velocity component Φ to which was added the displacement component Ψ .

t_1	$\Phi_3 - \Phi_2$	$\Phi_1 - \Phi_2 + \Phi_3$	Ψ	$\Psi + \Phi$
0	0	0	0	0
.2	1.51	22.86	3.4	26.26
.4	4.05	43.24	6.8	50.04
.6	7.13	61.26	10.3	71.56
.8	10.83	77.24	14.0	91.24
1.0	14.9	91.64	17.6	109.24
1.13	17.75	100.26	20	120.26
1.5	22.9	81.87	20	101.87
2	26.50	63.98	20	83.98
2.5	28.01	51.76	20	71.76
3	28.31	43.38	20	63.38
3.5	28.1	37.67	20	57.67
4	27.53	33.6	20	53.6
4.5	27.06	30.92	20	50.92
5	26.14	28.7	20	48.7
6	24.48	25.47	20	45.47
7	22.93	23.33	20	43.33
8	21.36	21.52	20	41.52
9	20.0	20.0	20	40
10	18.3	18.3	20	38.3
11	17.55	17.55	20	37.55
12	16.4	16.4	20	36.4
13	15.37	15.37	20	35.37
14	14.35	14.35	20	34.35
15	13.44	13.44	20	33.44
16	12.55	12.55	20	32.55

This sum $\Psi + \Phi$ was then plotted along with original observed response as shown in Fig.38.

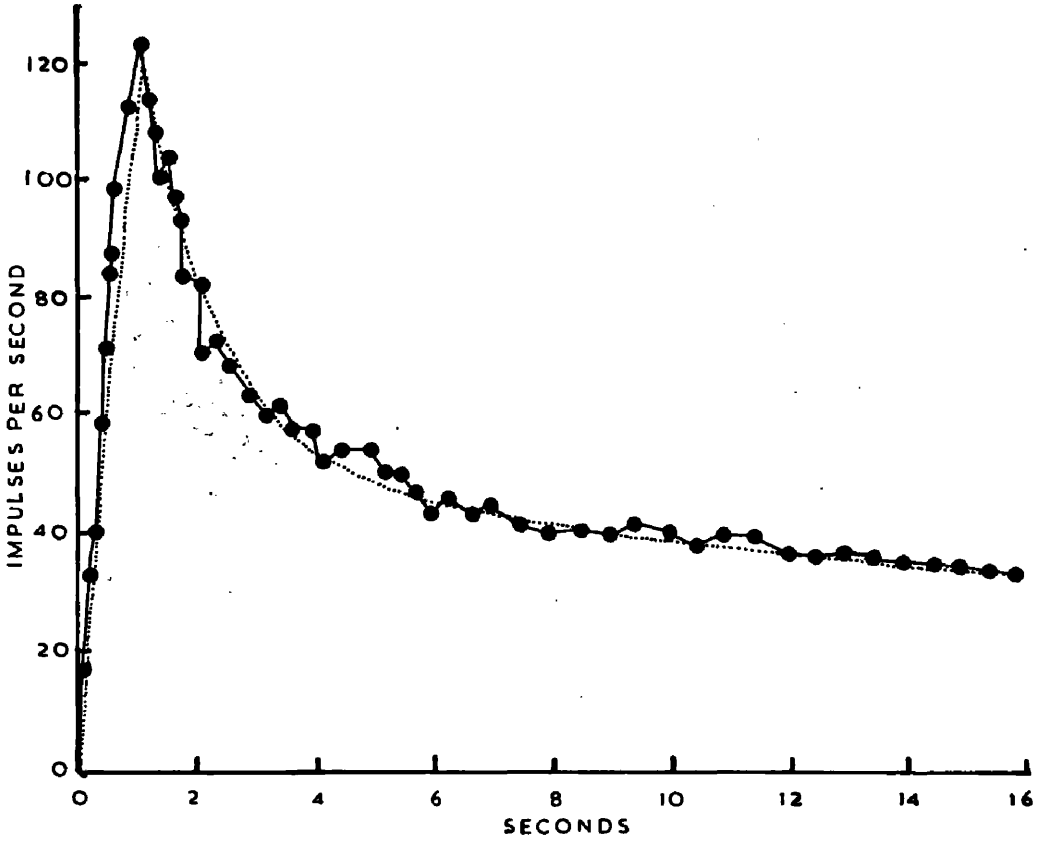

Fig.38. A comparison of the graphs of the observed and the calculated responses for unit 9(4)

● ——— ● observed response

..... calculated response.

Choice of parameters.

Six parameters $\phi_1, \phi_2, \phi_3, \tau_1, \tau_2, \tau_3$ have thus been determined for each constant velocity step. The question of the reliability of these values now arises. Are they at all critical, or would a wide range of values fit the experimental results equally well?

τ_3. The first parameter to be chosen is τ_3

The factors affecting the choice of τ_3 are:-

1) The value chosen for the static component, Ψ
2) The point on the discharge frequency curve after which Φ_3 is considered to act alone.
3) Alterations in the value chosen for Ψ will have a considerable effect on Φ_3

e.g. in unit 5C, an alteration of 2 in Ψ (when Ψ = 6)
——— 25% change in τ_3

in unit 7C, an alteration of 2 in Ψ (when Ψ = 10)
——— 50% change in τ_3

in unit 9(1), an alteration of 2 in Ψ (when Ψ = 20)
——— 20% change in τ_3

Since, in all units except 9, Ψ is low (about 10 impulses/sec.) it is liable to be irregular. Thus its determination is not very precise and hence no great reliability can be expected for the values of τ_3. They would be expected to vary quite a lot, and, in fact, do so.

The effect of variations in Ψ on the other parameters will be very much less, since variations in Ψ will tend to be compensated for by changes in the value of Φ_3 such that the total $\Psi + \Phi_3$ remains fairly constant. This is illustrated in Fig.39. Thus it might be expected that τ_3 and hence ϕ_3, would show greater variations than the other parameters. This is found to be true.

2) Another possible source of error in the determination of τ_3, would arise if, at the point X in Fig.30 either or both of the components Φ_1 and Φ_2 were still acting. In the determination of τ_3 it was assumed that at the point X on curve $F - \Psi$ the entire response was due to the component Φ_3

It seems likely however that any considerable error of this sort would be immediately apparent. For example, in the first analysis of one of the steps in unit 7, the point X was chosen to be about 5 seconds after the end of the movement. For this X, the value obtained for τ_3 led to values of Φ_3 which, in the earlier part of the response, were much greater than corresponding values of $F - \Psi$ (Fig.40).

When Ψ and the point X had been fixed, a value was chosen for τ_3 to fit the tail of the response curve. If the calculated component Φ_3 fitted the experimental curve reasonably well, the value chosen for τ_3 was considered to be satisfactory.

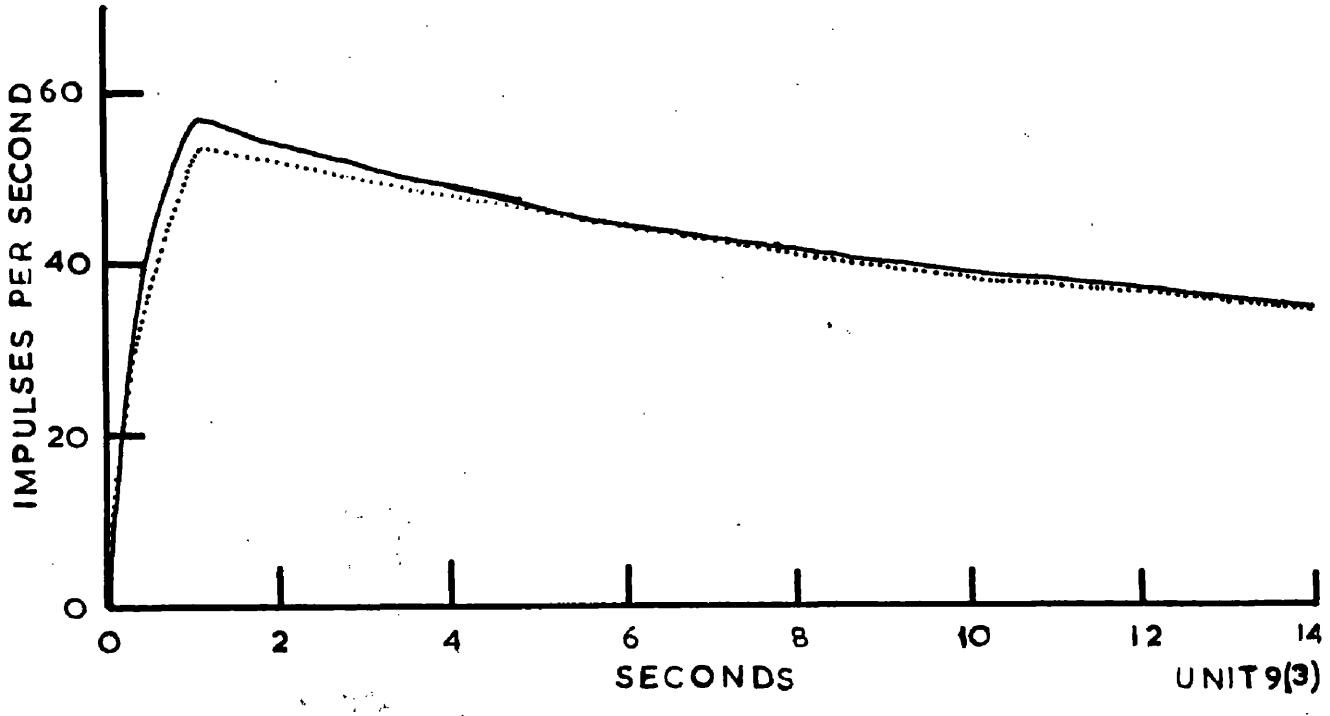

Fig.39. The effect of variations in the value of the displacement component $\Psi(s)$ on the sum $\Psi + \Phi_3$

——— $\Psi = 30, \quad \tau_3 = 8$

..... $\Psi = 20, \quad \tau_3 = 15.$

Variations in Ψ are to a large extent balanced by changes in τ_3 so that the sum $\Psi + \Phi_3$ varies little.

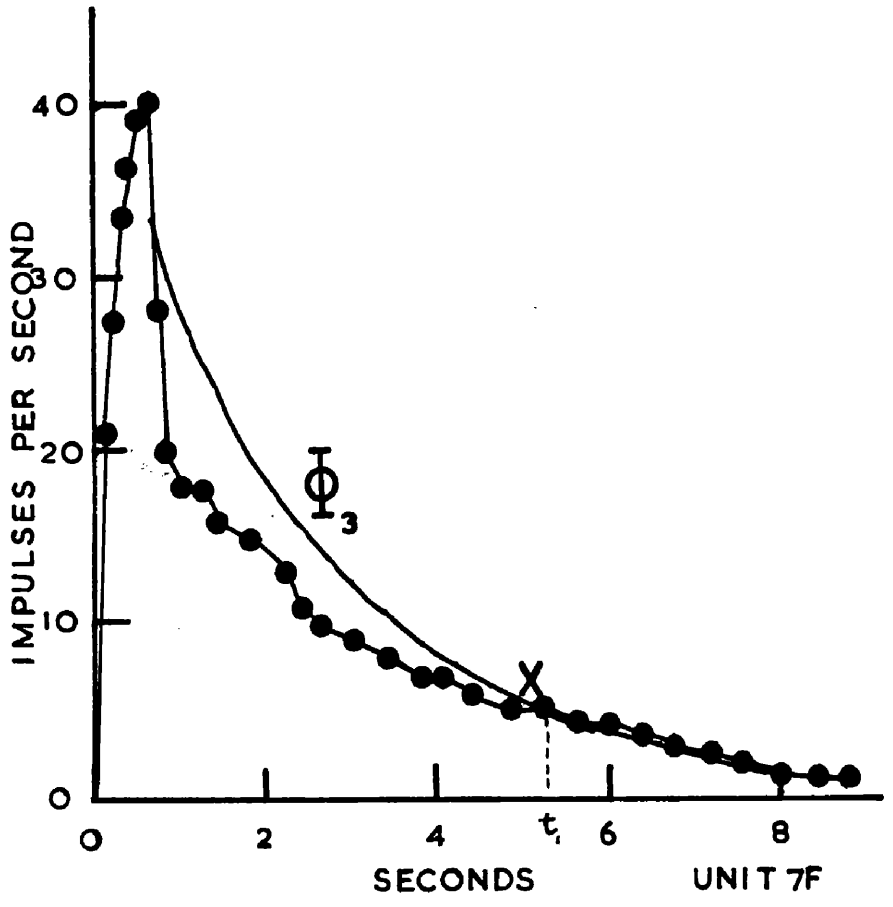

Fig.40. The effect of choosing the point X (when freq. is due solely to component Φ_3) too soon after the end of the movement.

A few calculations were made to see whether these values of τ_3 were at all critical or whether quite a wide range of values would fit the curve equally well. The results of these calculations are shown graphically in Fig.41.

The results of the alterations in τ_3 were:-

Unit 9	17% change in τ_3	——	little change in ϕ_3
	30% " " τ_3	——	worse fit of curve
Unit 5	25% " " τ_3	——	slightly worse fit of curves.

Thus it appears that values of τ_3 ± 20% of the chosen values would fit the experimental curves equally well. This wide range of possible values which might be chosen for τ_3 is due to the greater fluctuations at the tail of the experimental response, where the frequency is low

ϕ_3.

The value obtained for the parameter ϕ_3 is affected by the same factors as influence the choice of τ_3, and also by variations in the chosen τ_3

The changes shown in Fig.41 give the following changes in ϕ_3

	τ_3	ϕ_3		
Unit 9	12	15.9	i.e. 30% change in τ_3 ——	25% change in ϕ_3
	17	12.4		
	20	11.3	i.e. 18% change in τ_3 ——	8% change in ϕ_3
Unit 5	6	29	33% change in τ_3 ——	25% change in ϕ_3
	8	21		

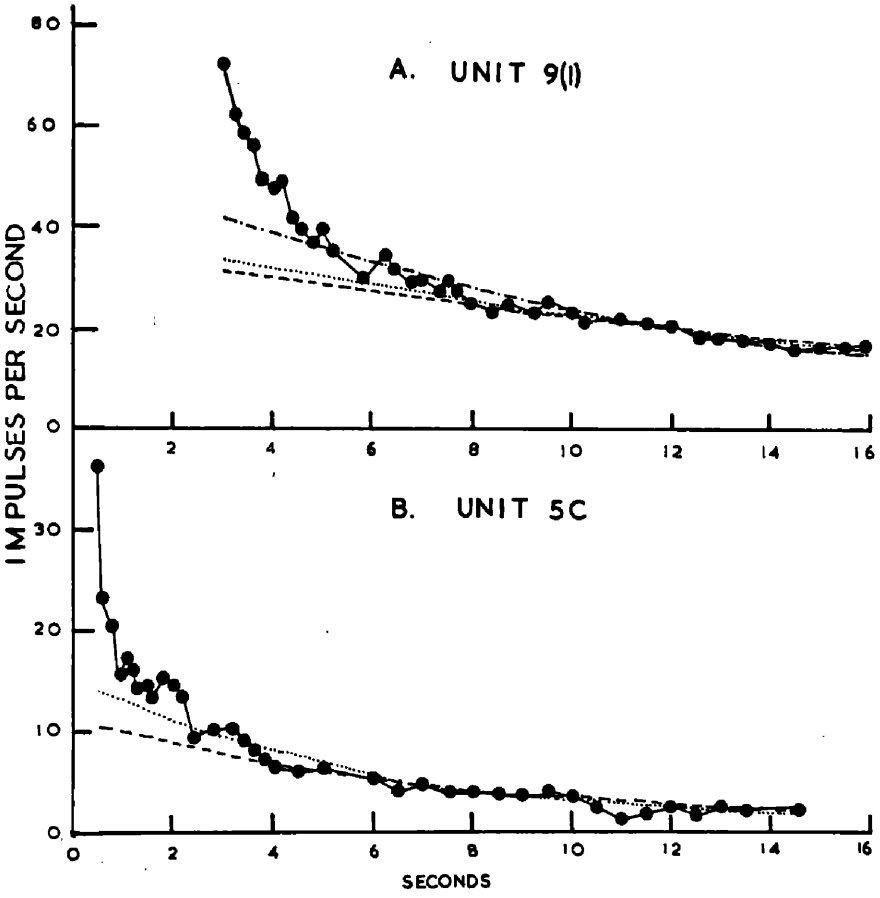

Fig.41. The effect of changes in the value chosen for τ_3 on the component Φ_3

A Unit 9 Observed values

Calculated values $\tau_3 = 12$

$\tau_3 = 17$

$\tau_3 = 20$

B Unit 5 Observed values

Calculated values $\tau_3 = 6$

$\tau_3 = 8$

$\phi, \phi_2, \tau, \tau_2$

The parameters ϕ and τ for the curves Φ_1 and Φ_2 are affected by

1) The shape of the curves Φ_1 and Φ_2

2) Errors in curve fitting.

Shape of Φ_1 and Φ_2

1). This is dependent on

a) Ψ

b) Φ_3

c) The curve C which is drawn by trial and error to give

$$\Phi_3 - C = \text{exponential}$$

$$\text{and } (F - \Psi) - C = \text{exponential.}$$

a) and b). Despite variations in Ψ and τ_3, the sum $(\Psi + \Phi_3)$ appears to remain fairly constant. This is illustrated in Fig.39. Thus it seems likely that variations in the values chosen for Ψ and Φ_3 will have little effect on the shape of Φ_1 and Φ_2.

c) Before attempting to draw the curve C, the curve $F - \Psi - \Phi_3$ was drawn to see if it was exponential in form. In only two cases could an exponential be fitted to it.

A number of different curves C were tried in each case, and that which gave Φ_1 and Φ_2 most nearly exponential was selected.

If a curve C could be found, such that

$$(F - \Psi) - C = \text{exp.} = \Phi_1$$

and $$\Phi_3 - C = \text{exp.} = \Phi_2$$

i.e., $$(F - \Psi) - \Phi_3 = \Phi_1 - \Phi_2$$

then Φ_1 and Φ_2 are unique,

since if $Ae^{ax} - Be^{bx} = Ce^{cx} - De^{dx}$

then $A = C \quad a = c$

$B = D \quad b = d$

2). Curve Fitting.

As with τ_3 , a few calculations were made to see if the values chosen for τ_1 and τ_2 were critical.

The results are shown graphically in Figs.42 and 43.

ϕ_1 and ϕ_2 are dependent on the values chosen for τ_1 and τ_2 and also on the peak values of Φ_1 and Φ_2

The changes in τ_1 and τ_2 shown in Fig.42 give the following changes in ϕ_1 and ϕ_2 :-

τ_1	ϕ_1		
1.2	42.7	i.e. 17% change in τ_1 ——	17% change in ϕ_1
1	49.5		
τ_2	ϕ_2		
1.4	6.7	i.e. 14% change in τ_2 ——	12% change in ϕ_2
1.2	7.5		

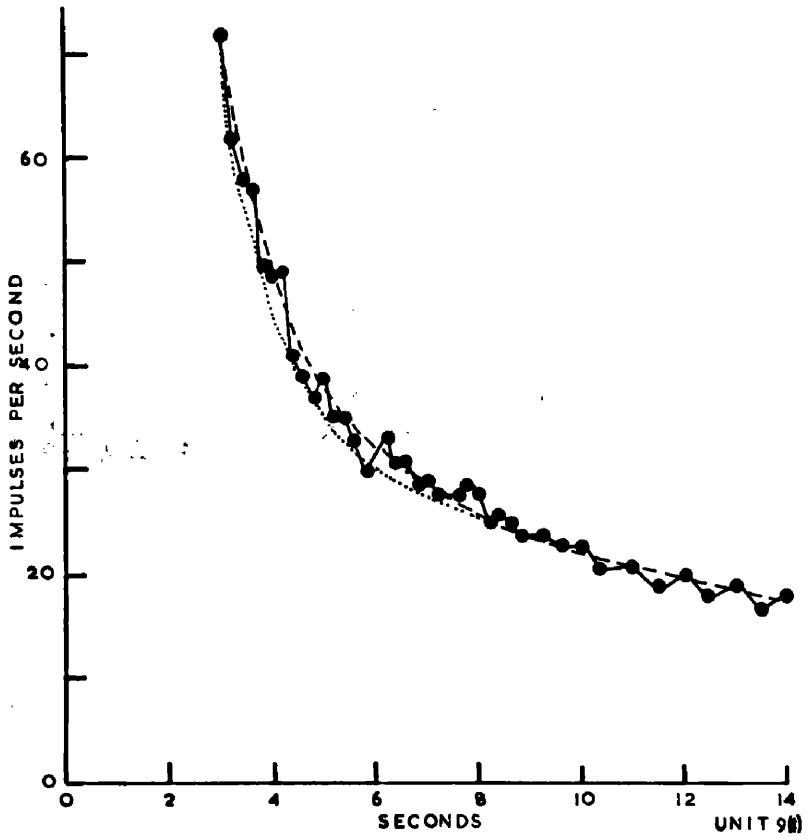

Fig.42. The effect on the total velocity component of changes in the value of τ_1

Observed response ●——●

Calculated response τ_1 = 1.2 sec. - - - - -

τ_1 = 1 sec. .....

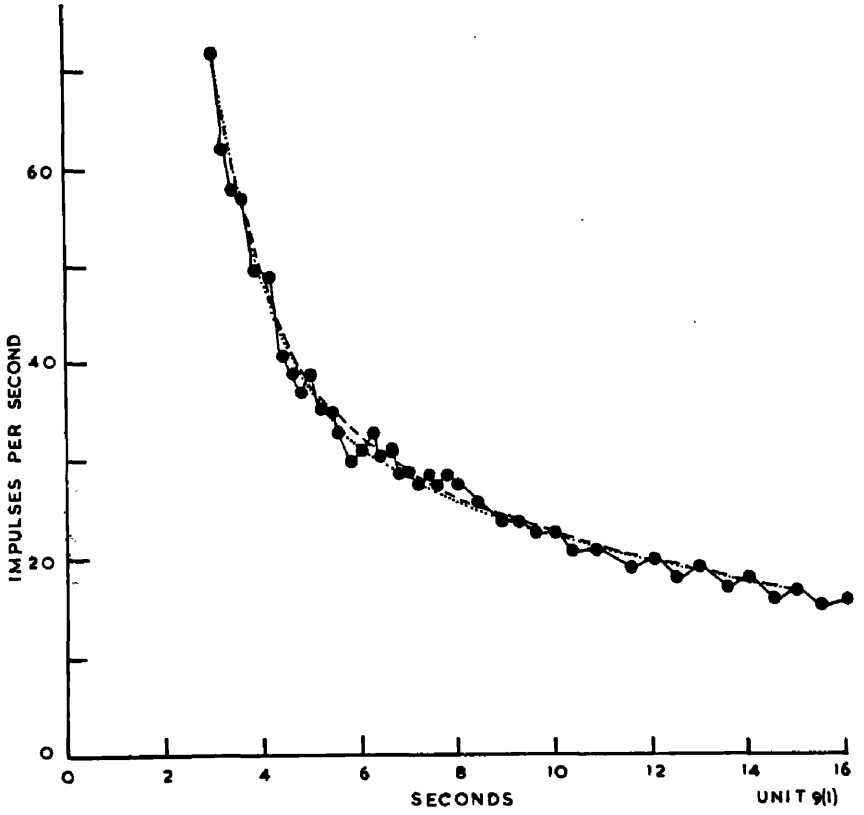

Fig.43. The effect on the total velocity component of changes in the value of τ_2

Observed response ● ——— ●

Calculated response τ_2 = 1.4 sec. - - - - -

τ_2 = 1.2 sec. .....

The parameters $\phi_1, \phi_2, \phi_3, \tau_1, \tau_2, \tau_3$ of all the analysed responses of a particular unit were collected (~~Table 6~~) and were plotted separately against v the velocity of the movement.

For each unit, it was found that the relationship between ϕ_i and v was approximately linear $\phi_i = k_i v$ ($i = 1, 2, 3$), where k is a constant which varied from unit to unit. The graphs ϕ_i / v for unit 9 are shown in Figs. 44 and 45.

When the parameters for the four units were plotted together (Figs.46 and 47) it was found that for units 5, 7, 8 $\phi_i = k_i v$ ($i = 1, 2, 3$).

No relationship between τ_i and v was found for any of the units. τ_1 and τ_2 were approximately constant, while τ_3 varied considerably (Fig.48).

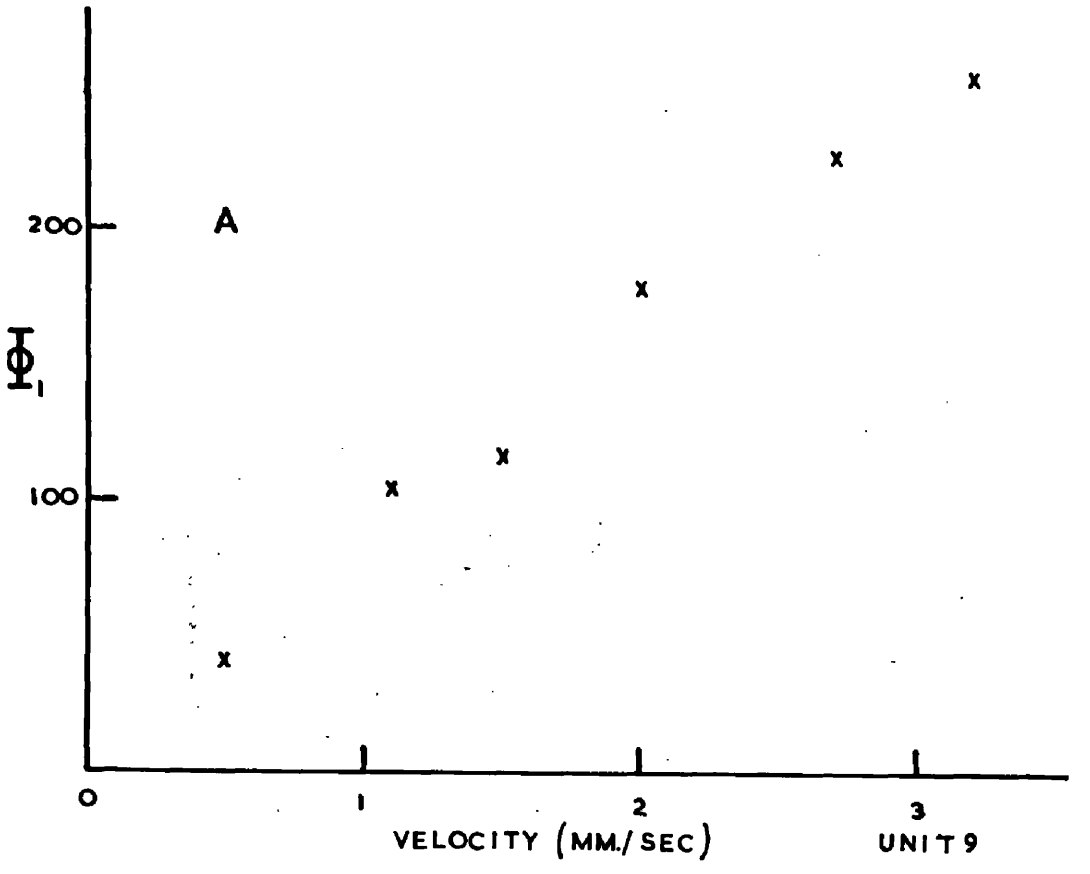

Fig.44. The relationship between the parameter Φ_1 and the velocity of the steps for unit 9.

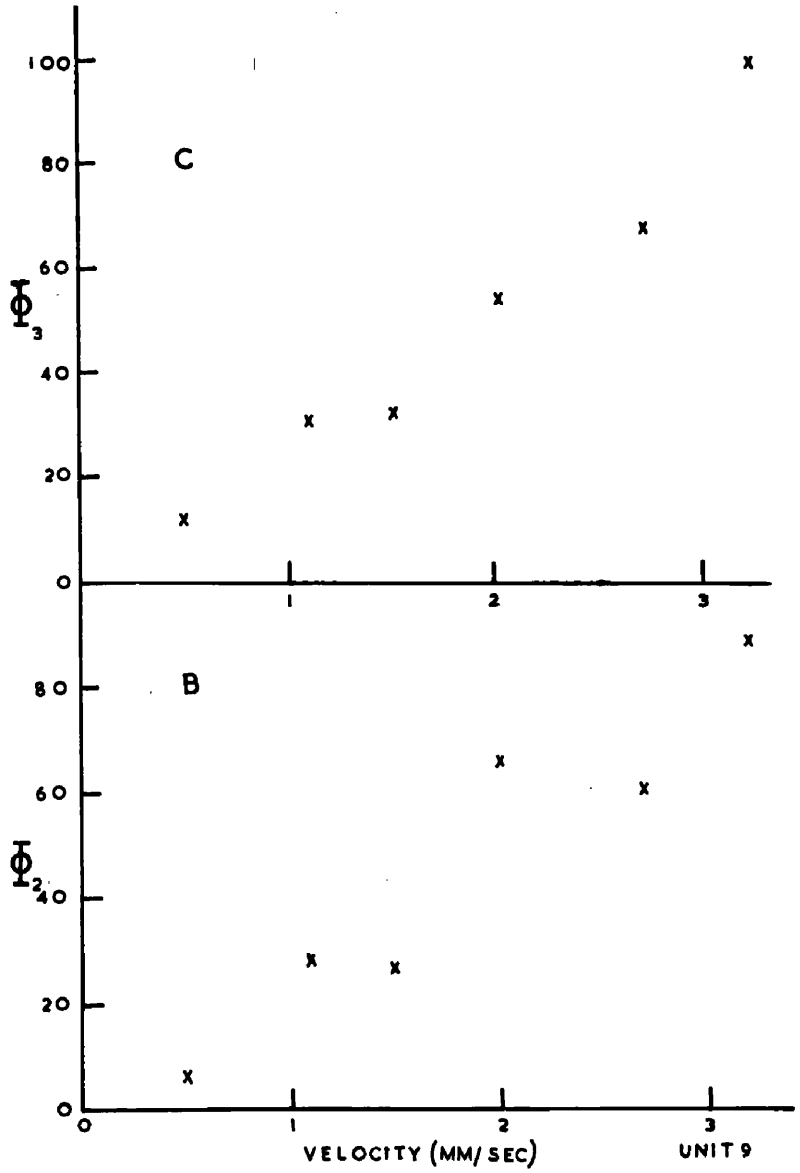

Fig.45. The relationship between the parameters ϕ_2 and ϕ_3 and the velocity of the steps for unit 9.

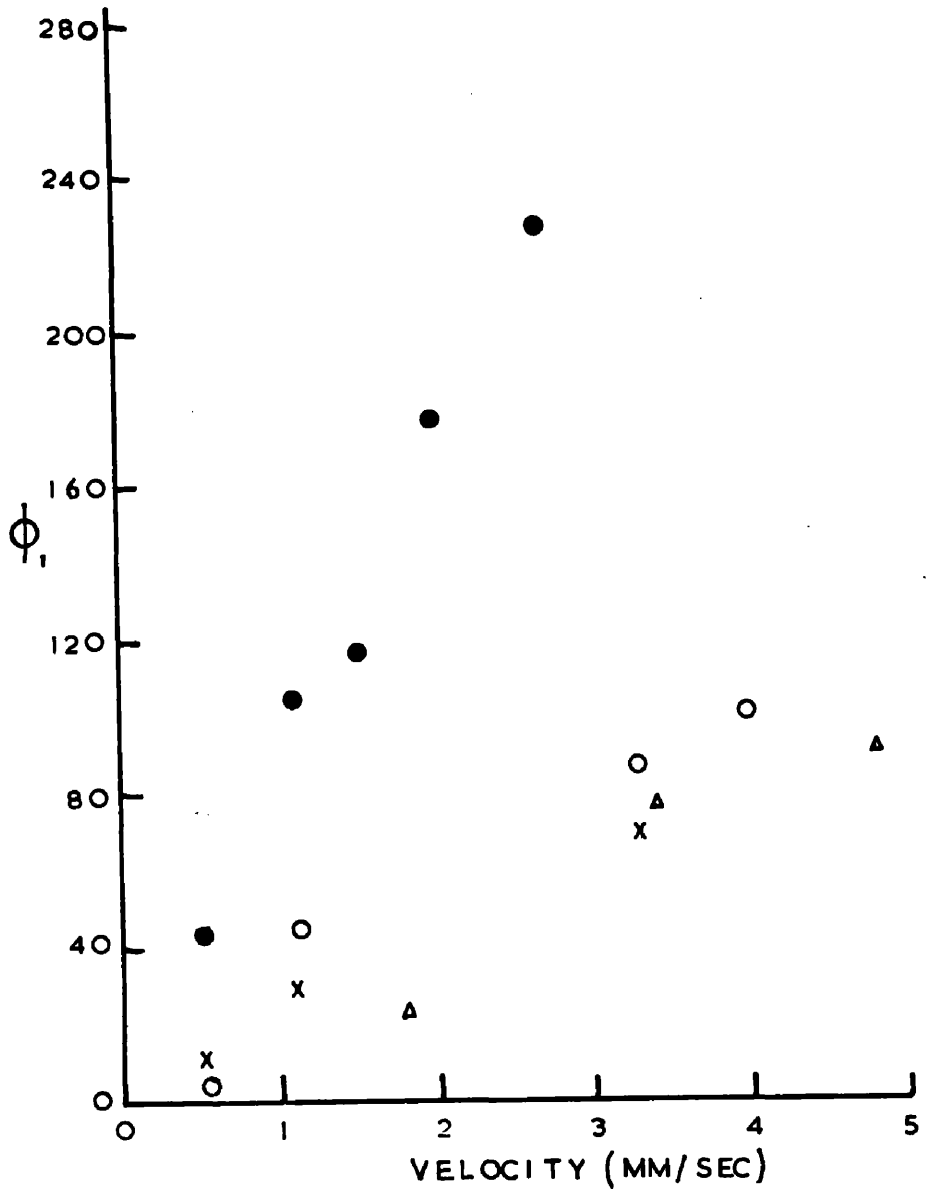

Fig.46. The relationship between the parameter ϕ_1, and the velocity of the steps for units 5, 7, 8 and 9.

△ Unit 5

○ Unit 7

× Unit 8

● Unit 9.

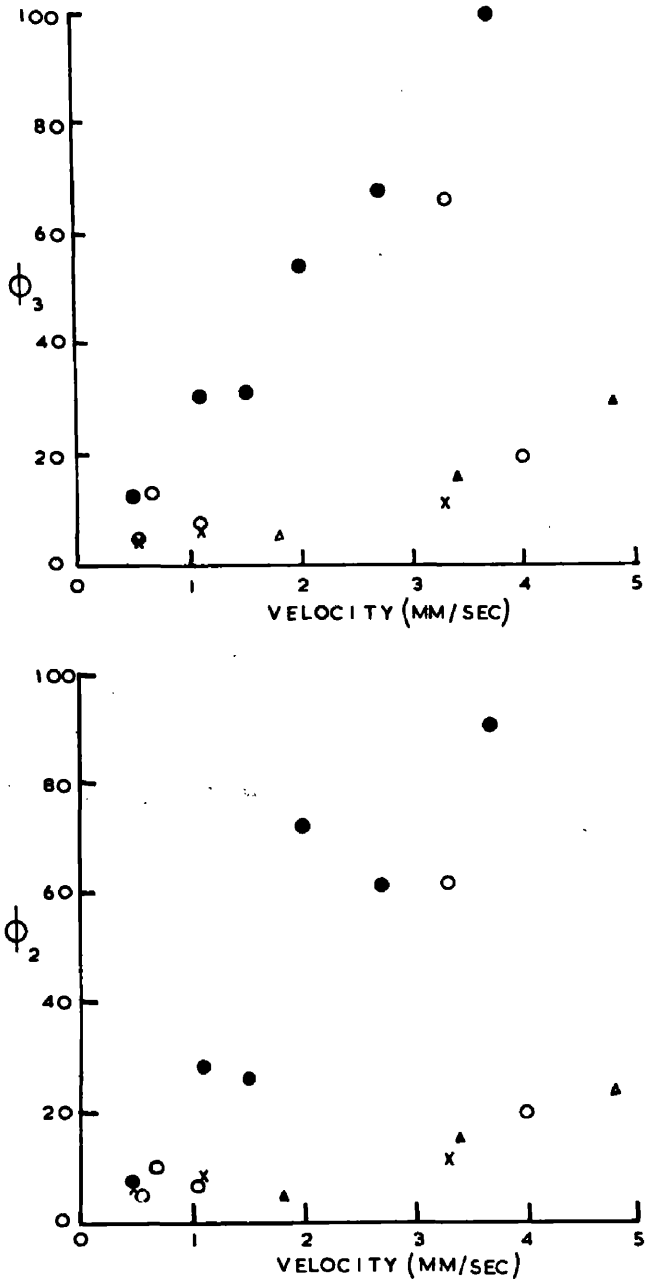

Fig.47. The relationship between the parameters Φ_2 and Φ_3 and the velocity of the steps for units 5, 7, 8 and 9.

△ Unit 5

○ Unit 7

× Unit 8

● Unit 9.

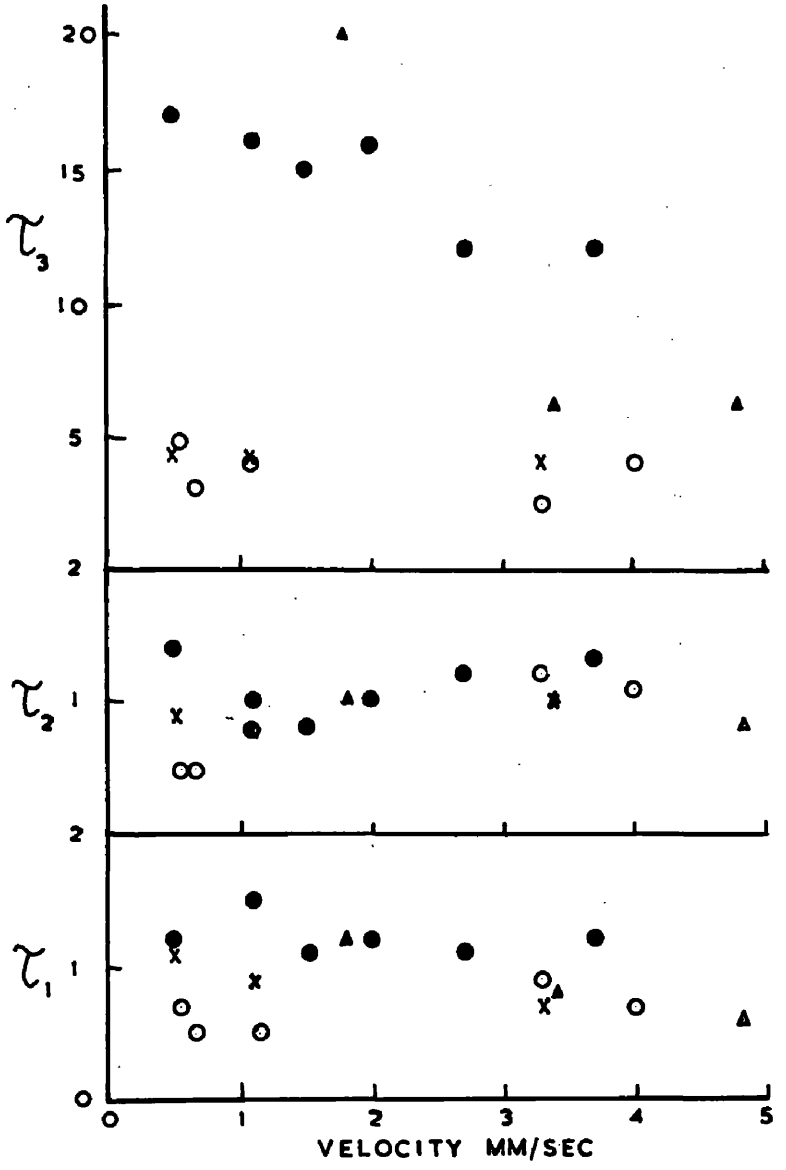

Fig.48. The relationship between the parameters τ_i (i = 1, 2, 3) and the velocity of the steps for units 5, 7, 8 and 9.

△ Unit 5

○ Unit 7

× Unit 8

● Unit 9.

Time of Movement.

Some calculations were made to see what effect errors in the measurement of t'', the duration of the movement, would have on the calculated response.

This error would be most likely to occur in a slow movement when, because of the low gradient, the exact moments when the transducer signal left, and returned to, the base-lines, would be rather difficult to determine.

In unit 9(1), which was a slow velocity step, t'' was changed from 3 to 2.5 sec. The effect on the calculated response after the end of the movement is shown in Fig.49 and was found to be negligible.

This alteration in the value of t'' leads to a change in the values of ϕ_1 ϕ_2 and ϕ_3. However, the velocity v is also altered, and the new values of ϕ_i remain on the line $\phi_i = k_1 v$.

In unit 5, the ending did not start to fire until some way through the movement, so that the time of stimulation was not the same as the time of movement. (In this unit the relationship between ϕ and v can be best expressed $\phi = -f + kv$, perhaps reflecting the below threshold state of the ending.)

The analysis of unit 5C was carried out using $t'' = 0.4$ sec. (duration of stimulation) instead of $t'' = 0.5$ sec. (duration of movement). The results are shown in Fig.50. The value of the total velocity component was practically unaltered.

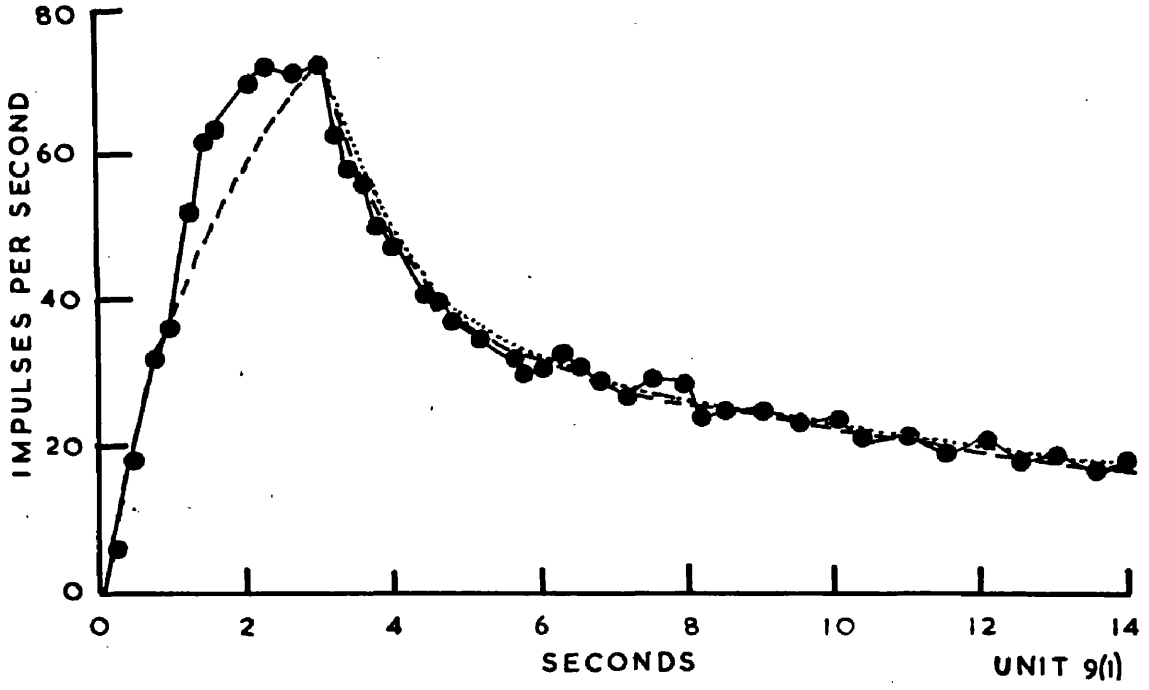

Fig.49. The effect on the calculated response frequency after the end of the movement, of altering the value of t", the duration of the movement.

Observed response ●——

Calculated response t" = 3 sec. - - - - -

t" = 2.5 sec. .....

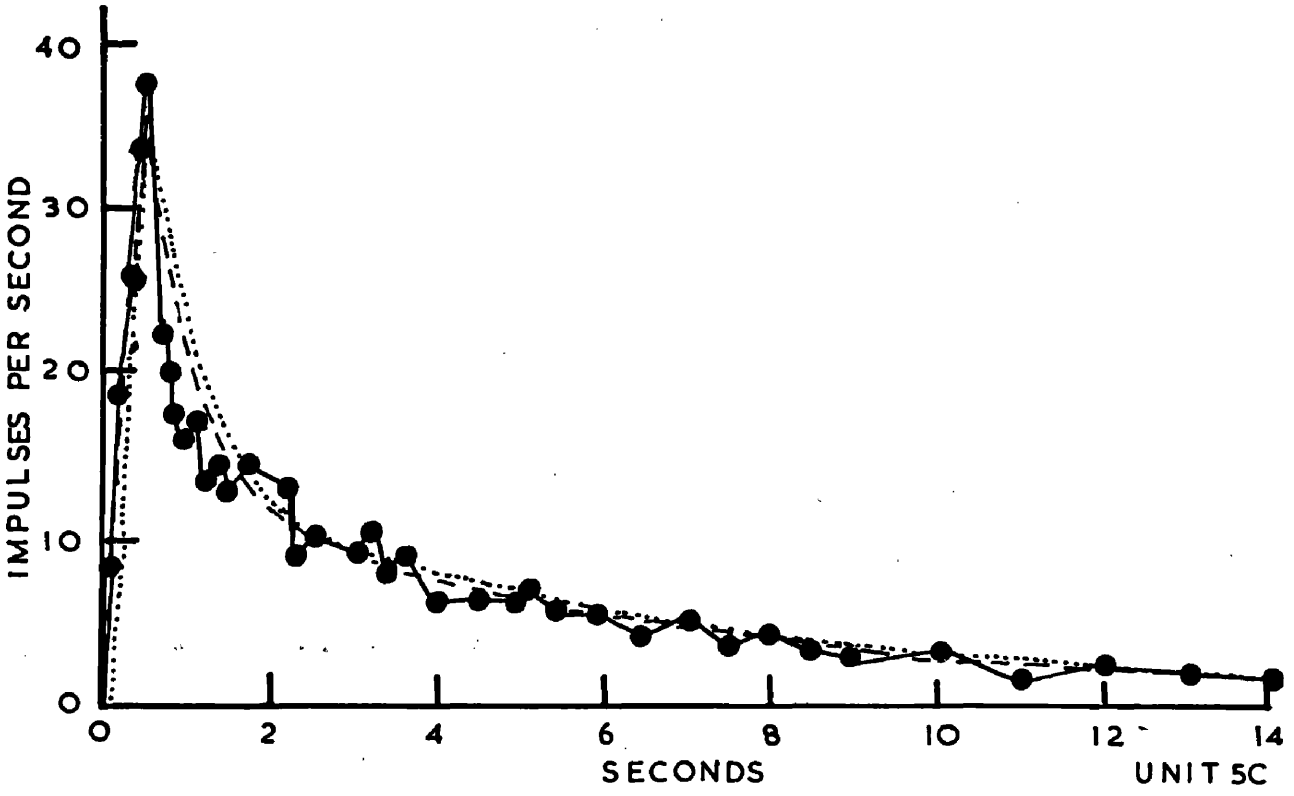

Fig.50. The effect on the calculated response frequency after the end of the movement, of calculating the response using t'' as duration of stimulation instead of duration of movement.

Observed response ●——●

Calculated response t'' = duration of movement - - - -

t'' = duration of stimulation .....

Response to Sinusoidal Movement.

The real test of the validity of the application of the transfer function to the frog neuromuscular spindle was to see whether the parameters, derived from the constant velocity steps, could be used to calculate the expected response to sinusoidal movement with any accuracy.

On all the occasions when the muscle was stretched with steps of constant velocity, sinusoidal extension and relaxation were also applied (Table 3).

Using the apparatus described above, the cord from the puller was attached to the driving shaft of the S.H.M. machine. The point of attachment was such that the extension of the muscle was over the same range as was used for the corresponding constant velocity steps. The S.H.M. machine was adjusted to give its slowest speed and the response to three successive cycles recorded. This procedure was repeated at the other two chosen speeds. These had been previously selected to give as wide a frequency range as possible without changing the driving belt.

The responses to these sinusoidal movements were considered in detail in the same four units in which the responses to steps of constant velocity had been analysed, and the parameters of the transfer function derived, viz, units 5, 7, 8 and 9.

Table 3.

Table showing those units which were stretched sinusoidally and those in which the response to this movement was compared with the predicted response, calculated using the transfer function.

Unit	Range (mm.)	Speed c/sec.	No. of cycles	Response measured	Expected response calculated using transfer function
1				No (Film speed too slow)	
2				No (Freq. very low)	
3	2.4	.22	3	+	No parameters from steps
		.3	3	+	
		.4	3	+	
	4	.2	3	+	
		.3	3	+	
		.4	3	+	
4	2			No (Movement not purely sinusoidal)	
5	2.4	.2	3	+	+
		.3	3	+	+
		.4	3	+	+
	1.6	.2	3	+	-
		.3	3	+	-
		.4	3	+	-
6	2	.2	3	+	No parameters from steps
		.3	3	+	
		.4	3	+	
7	2.4	.2	3	+	+
		.3	3	+	+
		.4	3	+	+
8	2	.2	3	+	+
		.3	3	+	+
		.4	3	+	+
9	1.6	.2	3	+	+
		.3	3	+	+
		.4	3	+	+
	2.8	.2	3	+	-
		.3	3	+	-
		.4	3	+	-

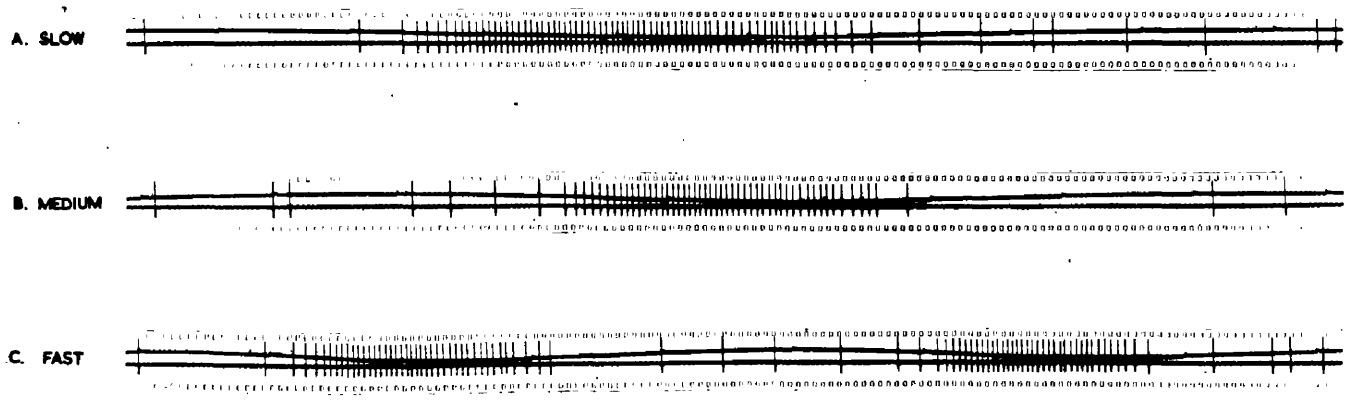

Fig.51. Oscillograph records of the effect on the sensory discharge of the application of sinusoidal movements (Unit). The upper beam signals the movement and also carries the time-marker ($\frac{1}{2}$ sec. pulses).

The impulse frequency recorded in response to the sinusoidal movement was plotted. The point reached in the extension cycle was used as abscissa, instead of time, as in the graphs of the response to the constant velocity steps. This method meant that the responses to sinusoidal movements of different speeds were comparable and that the effect on the response of changes in speed could be readily appreciated.

For each speed used the plots of repeated cycles were superimposed. In units where the maximum response frequency was low, around 30 impulses/sec., the response to stretch was rather irregular and consequently varied between successive cycles (Fig.52A). When the maximum frequency was about 60 impulses/sec., the response was much more regular and successive cycles gave very similar responses (Fig.52B).

A mean curve was drawn through the superimposed responses to successive cycles. For each unit the mean curves for the three different speeds used were then plotted together (Fig.53).

Increase in the speed of the sinusoidal movement, for the range used in these experiments, produces no very striking changes in the response: however, the following observations can be made:-

1. The maximum frequency of the response increases with speed.
2. As speed increases, this peak frequency occurs at an earlier point in the cycle.

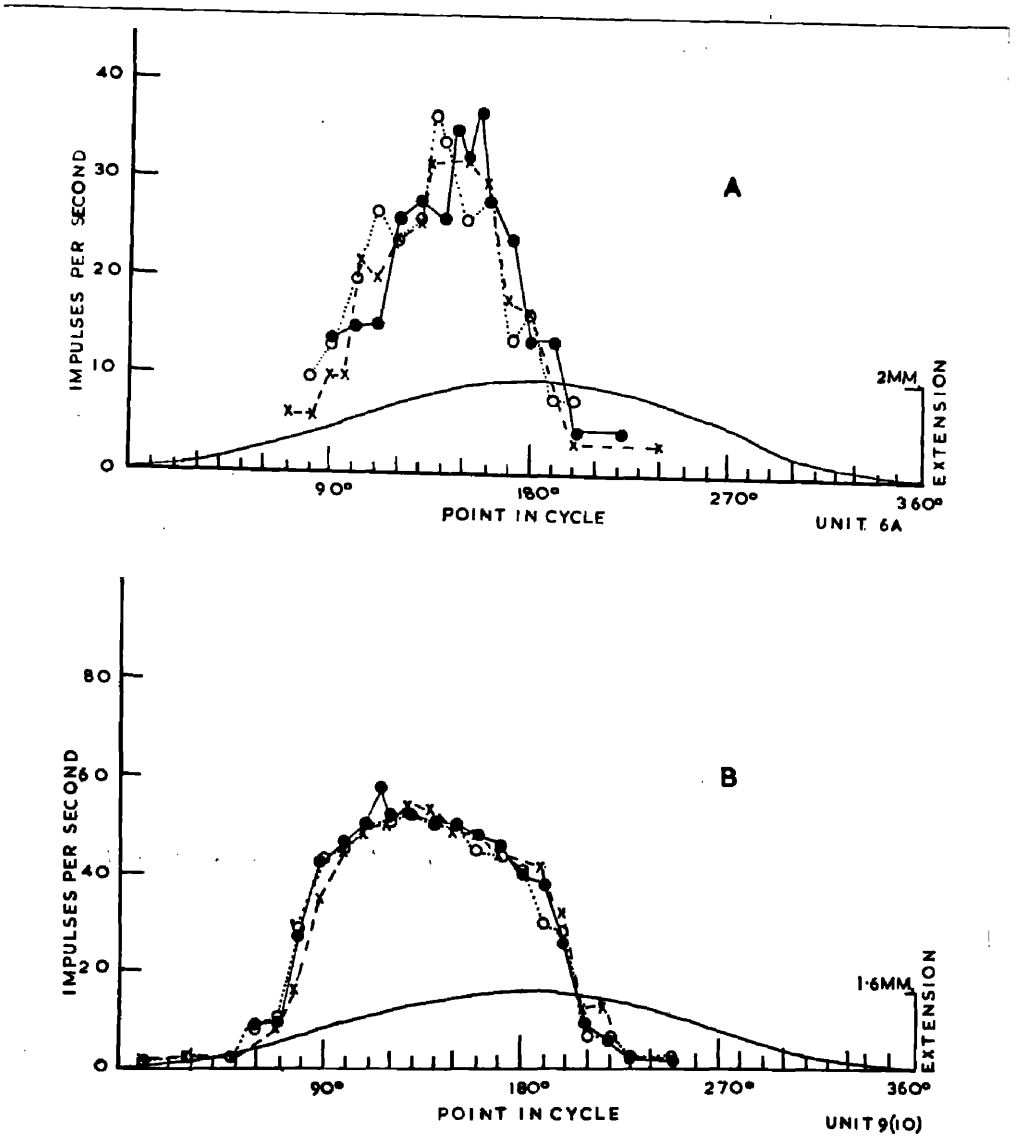

Fig.52. Graphs of responses to successive cycles at the same speed.

● —— ● 1st cycle
× – – – × 2nd cycle
⊙ ····· ⊙ 3rd cycle.

A Maximum frequency of response ~ 30 impulses/sec.
B Maximum frequency of response ~ 60 impulses/sec.

The unit which has the higher response frequency has the more regular discharge.

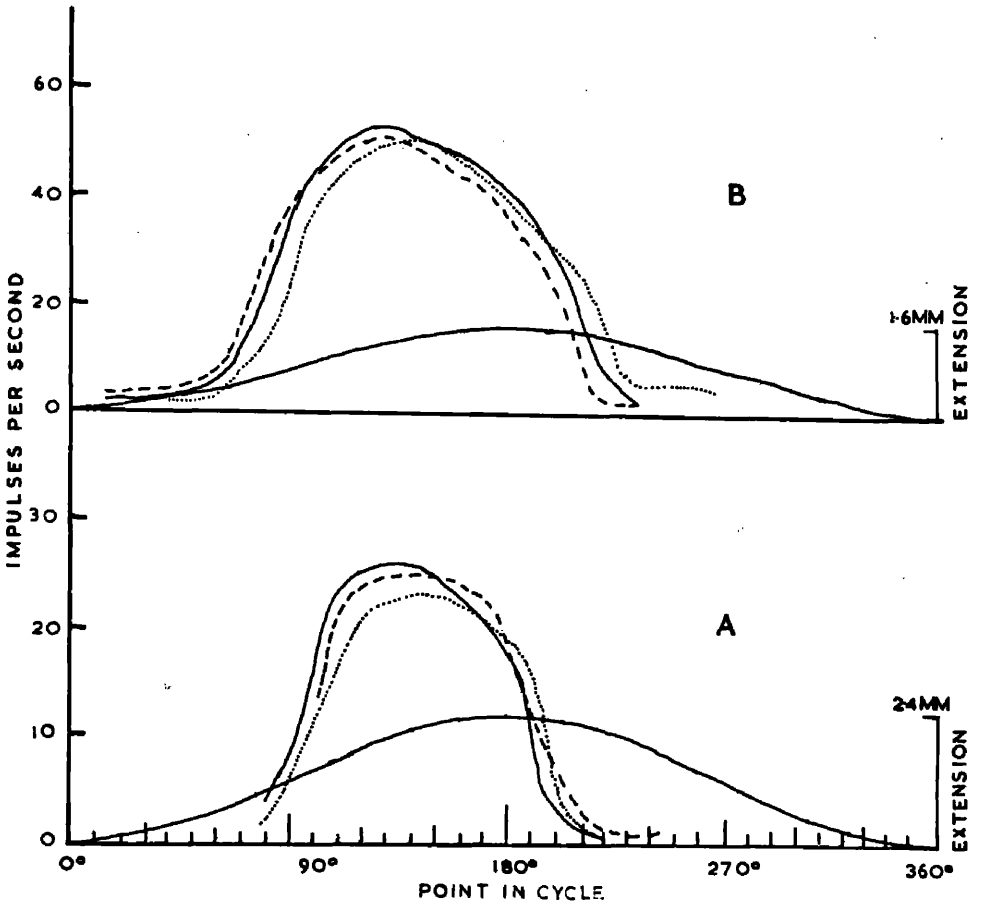

Fig.53. The effect on the response of changes in the speed of the sinusoidal movement.

Average responses at three different speeds

..... slow (0.2 cycles/sec.)
- - - - - medium (0.3 cycles/sec.)
——— fast (0.4 cycles/sec.).

A Unit 5

B Unit 9 (note different scale in ordinate).

For each unit the expected responses to sinusoidal movements of the three different speeds used was calculated by means of the transfer function, using the parameters derived from the steps. The results of these calculations were compared with the observed responses.

From the analysis of the responses to steps of constant velocity, the parameters $\phi_1, \phi_2, \phi_3, \tau_1, \tau_2 \cdot \tau_3$ were determined for each of the different constant velocity steps applied to a unit.

For each unit ϕ_i (i = 1,2,3) was plotted against v. As shown above, it was found that the relationship between them was almost linear, giving $\phi_i = k_i v$ (i = 1,2,3). The constants k_i were determined.

τ_i was approximately constant, for any unit, and the average values were selected.

The parameters, thus chosen, were used to calculate the expected response to the sinusoidal movement.

From the theory, given above, at angular velocity w and time t_1,

The component of frequency $\Phi_i(\omega) = C\left[\frac{1}{\tau_i}\sin\omega t_1 - \omega\cos\omega t_1\right]$

due to velocity, i = 1,2,3

where $C = \frac{ak_i}{\omega^2 + \frac{1}{\tau_i^2}}$ and a = rw

$$\therefore \quad \Phi_i(\omega) = \frac{r\omega k_i}{\omega^2 + \frac{1}{\tau_i^2}}\left[\frac{1}{\tau_i}\sin\omega t_1 - \omega\cos\omega t_1\right]$$

The total component $= \Phi_1 - \Phi_2 + \Phi_3$

due to velocity

$$= r\omega\left[\frac{k_1}{(\omega^2+\frac{1}{\tau_1^2})\tau_1} - \frac{k_2}{(\omega^2+\frac{1}{\tau_2^2})\tau_2} + \frac{k_3}{(\omega^2+\frac{1}{\tau_3^2})\tau_3}\right]\sin\omega t_1$$

$$- r\omega^2\left[\frac{k_1}{\omega^2+\frac{1}{\tau_1^2}} - \frac{k_2}{(\omega^2+\frac{1}{\tau_2^2}} + \frac{k_3}{\omega^2+\frac{1}{\tau_3^2}}\right]\cos\omega t_1$$

$$= A \sin wt_1 + B \cos wt_1$$

Now the function $A \sin\theta + B \cos\theta$ has a maximum value $A^2 + B^2$ when $\theta = \tan^{-1}\frac{B}{A}$

$\therefore$ Maximum value of velocity component $A \sin wt_1 + B \cos wt_1$

$$= A^2 + B^2$$

which occurs when $wt_1 = \tan^{-1}\frac{B}{A}$

where $A = r\omega\left[\frac{k_1}{(\omega^2+\frac{1}{\tau_1^2})\tau_1} - \frac{k_2}{(\omega^2+\frac{1}{\tau_2^2})\tau_2} + \frac{k_3}{(\omega^2+\frac{1}{\tau_3^2})\tau_3}\right]$

and $B = -r\omega^2\left[\frac{k_1}{\omega^2+\frac{1}{\tau_1^2}} - \frac{k_2}{\omega^2+\frac{1}{\tau_2^2}} + \frac{k_3}{\omega^2+\frac{1}{\tau_3^2}}\right]$

The coefficients A and B were calculated for each unit, for the three different speeds used. The maximum response frequencies and their points of occurrence in the cycle were determined. The displacement component Ψ (S), at these points, was then added to the maximum values of the response and the calculated peak frequencies compared with the experimental values. If the calculated values agreed with the experimental ones to within about $\pm$ 10%, the

theoretical response was calculated for the complete cycle.

In no case did the calculation, using the parameters derived from the steps, give an exact fit of the experimental curves of the response to sinusoidal motion. Fig.54 shows the observed and calculated responses of unit 7 at the three speeds used and compares them at the fastest speed.

In general, the response calculated using the transfer function agreed more closely with the experimental response at the lower rather than the faster speed.

The differences between the two responses are:-

1) With increase in speed the experimental response shows a greater increase than the theoretical one.
2) With increase in speed the maximum frequency occurs
   a) experimentally - at earlier point in cycle,
   b) theoretically - at later point in cycle.
3) Experimental response departs much more from a sinusoidal form than the theoretical one does.

The question now arises as to whether these discrepancies are due solely to a bad choice of parameters for the transfer function, or whether some other factors should have been taken into consideration in estimating the theoretical response.

The values chosen for the parameters were by no means precise; the τ's showed quite a broad scatter and the

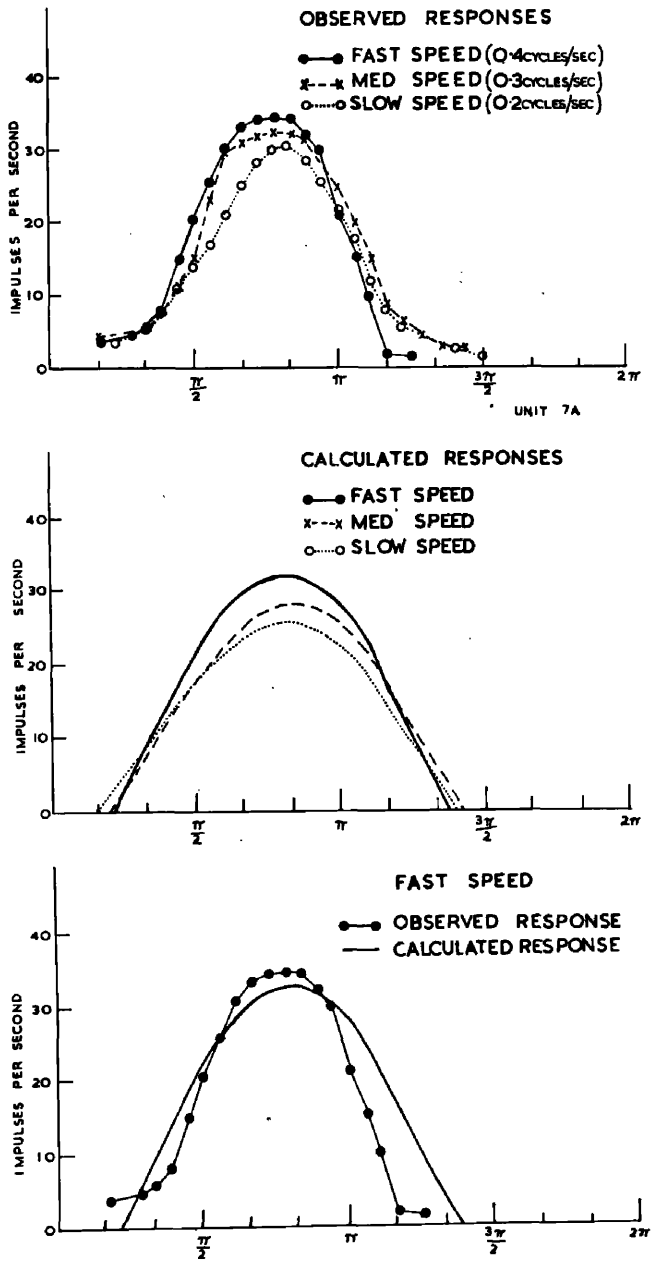

Fig.54. Graphs of the observed and the calculated responses to sinusoidal movements at 3 different speeds.

Upper fig. Observed responses.

Middle fig. Calculated responses.

Lower fig. Comparison of the observed and calculated responses at the fastest speed.

gradient k_i of the lines $\phi_i = k_i v$ could also take a range of values.

The effect on the response of various changes in the values of the parameters τ_i and k_i was therefore tried and the results shown in Figs.55-60 and Table 4.

These changes can be summarised.

Parameter	Change	Number of units in which change was made	Effect on a) Peak freq.	b) Lag
τ_1	decrease	4	Dec.	Dec.
τ_2	"	1	No change	
τ_3	"	1	Inc.	Dec.
k_1	increase	2	Inc.	Dec.
k_2	"	2	Dec.	No change
k_3	"	1	Inc.	Inc.

The desired changes in the form of the calculated response to give closer agreement with the observed response are:-

a) Increase in peak frequency.

b) Decrease in lag, i.e., in that part of the cycle which elapses between the point of maximum velocity and the point of occurrence of the maximum response frequency.

These changes could be achieved by:

a) Decrease in τ_3 Increase in k_1 and k_3.

b) Decrease in τ_1 and τ_3 Increase in k_1. (The decrease in τ_1 has the greatest effect.).

Table 4.

The effect of alterations in the values of the parameters τ_1, τ_2, τ_3, k_1, k_2 and k_3 on the calculated values of the peak frequency and the lag of the response to sinusoidal movements at the three speeds (S,M,F).

Parameter	Change	Unit	Peak Freq. Change	Peak Freq. Previous max. (Imp/sec)	Peak Freq. Alteration (Imp/sec) (S,M,F)	Lag Change (Degrees) (S,M,F)
τ_1	Decrease 20%	5	Decrease	25	(2,2,2)	Dec. (10,10,10)
	" 40%	5	"	25	(4,4,4)	" (15,15,15)
	" 30%	7	"	32	(6,5,2)	" (10,10,0)
	" 10%	8	"	27	(1,2,2)	" (3,10,10)
	" 30%	9	"	50	(12,12,10)	" (5,5,10)
τ_2	Decrease 10%	7	No change			No change
	" 20%	7	Increase	27	(1,2,1)	Incr. (0,5,0)
τ_3	Decrease 30%	7	Increase	25	(4,1,0)	Dec. (0,5,5)
	" 30%	7	No change			No change
	" 60%	7	"			Dec. (5,5,0)
k_1	Decrease 10%	5	Decrease	18	(2,2,2)	No change
	" 10%	7	"	30	(3,3,3)	Incr. (10,10,10)
	" 15%	7	"	30	(4,4,4)	Incr. (10,10,10)
k_2	Decrease 10%	5	Increase	17	(1,1,1)	No change
	" 30%	5	"	17	(2,2,2)	"
	" 20%	7	No change			Incr. (5,5,5)
k_3	Decrease 16%		Decrease	17	(1,1,1)	No change

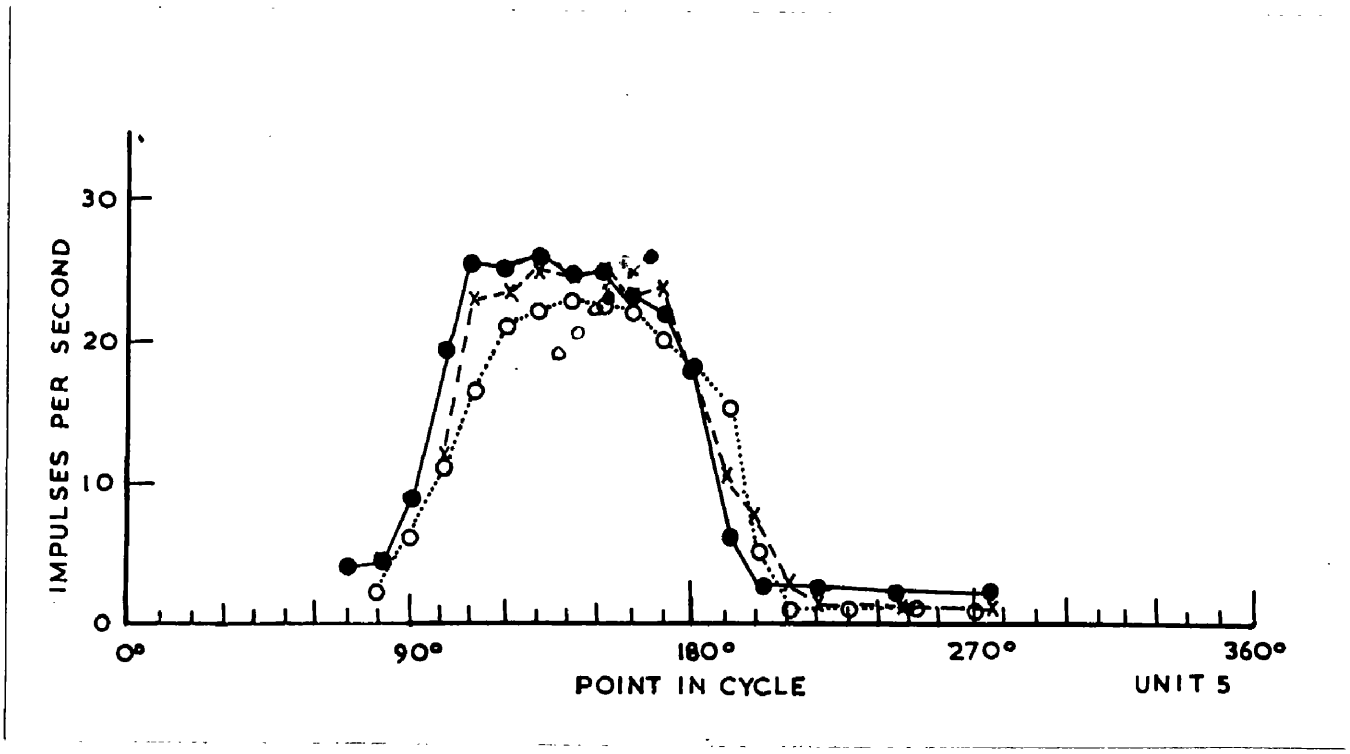

Fig.55. The effect of changes in the value of τ_1 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values

o·····o slow speed
x-----x medium speed
●——● fast speed

Calculated peak values

	τ_1	τ_2	τ_3
o slow ● fast	.6	1.2	7
o slow x med. ● fast	1	1.2	7
o slow x med. ● fast	.8	1.2	7

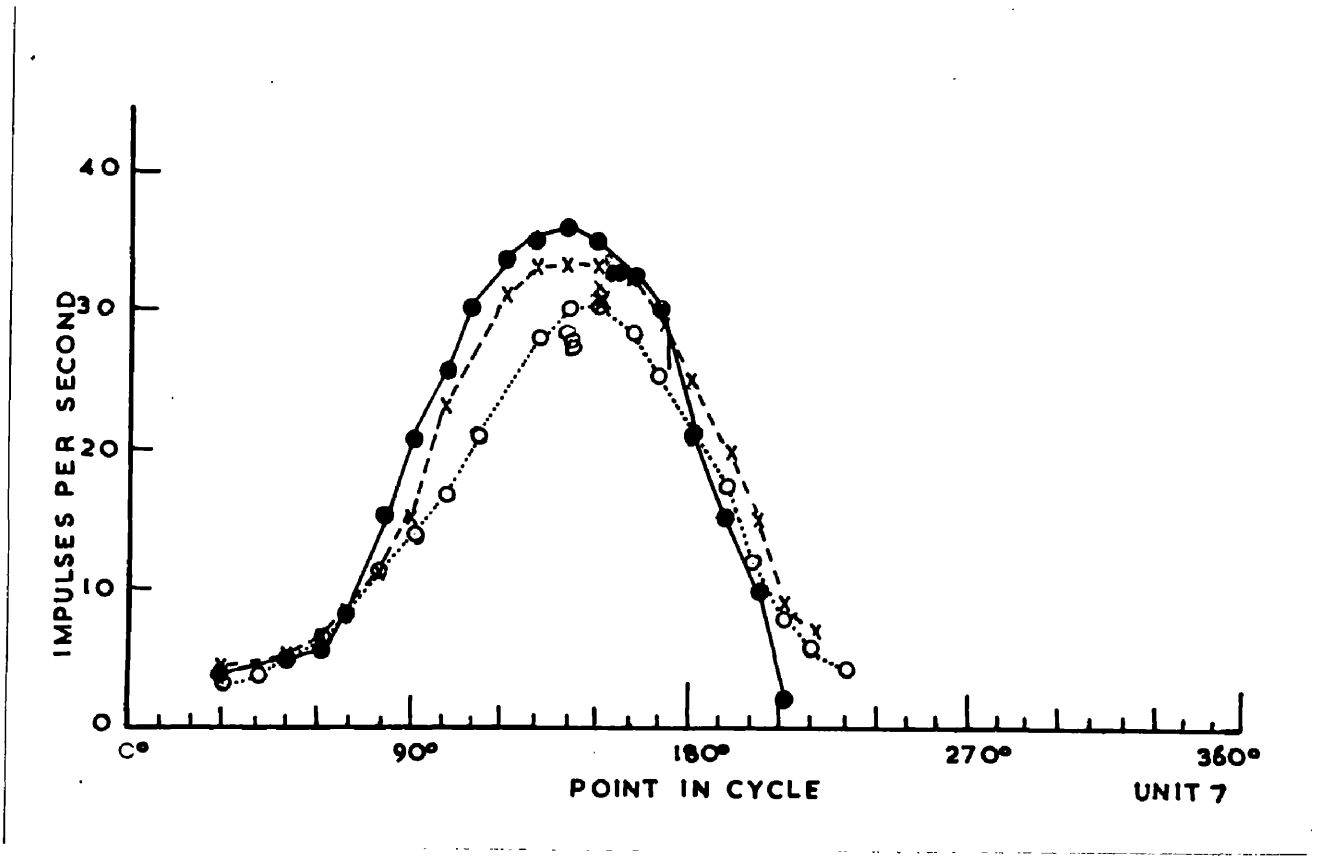

Fig.56. The effect of changes in the value of τ_2 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values

o ····· o slow speed
× - - - - × medium speed
● —— ● fast speed.

Calculated peak values		τ_1	τ_2	τ_3
o slow × med. ● fast		.6	.9	4
o slow X med. ● fast		.6	1	4
o slow × med. ● fast		.6	.8	4

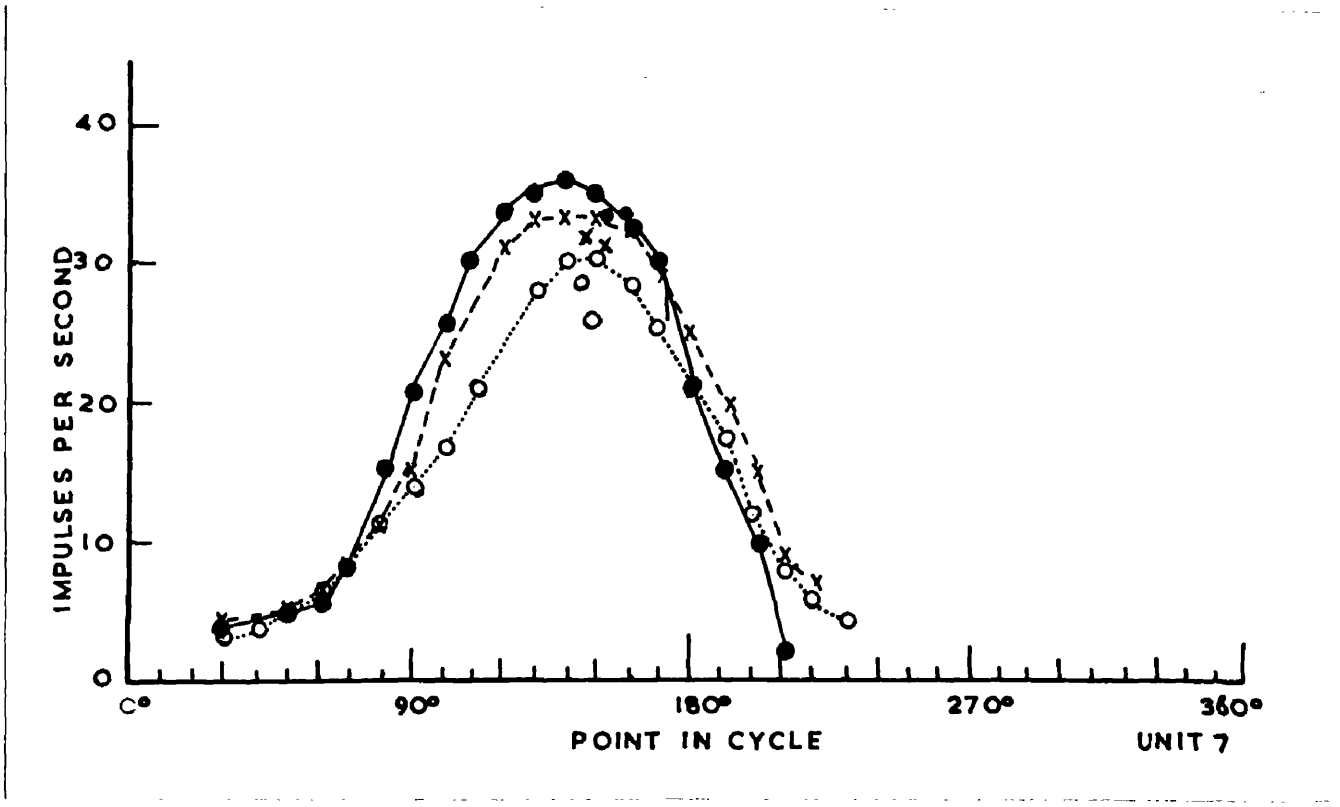

Fig.57. The effect of changes in the value of τ_3 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values
o·····o slow speed
×----× medium speed
●——● fast speed.

Calculated peak values

		τ_1	τ_2	τ_3
o	slow	.6	.8	3
×	med.			
●	fast			
o	slow	.6	.8	2
×	med.			
●	fast			

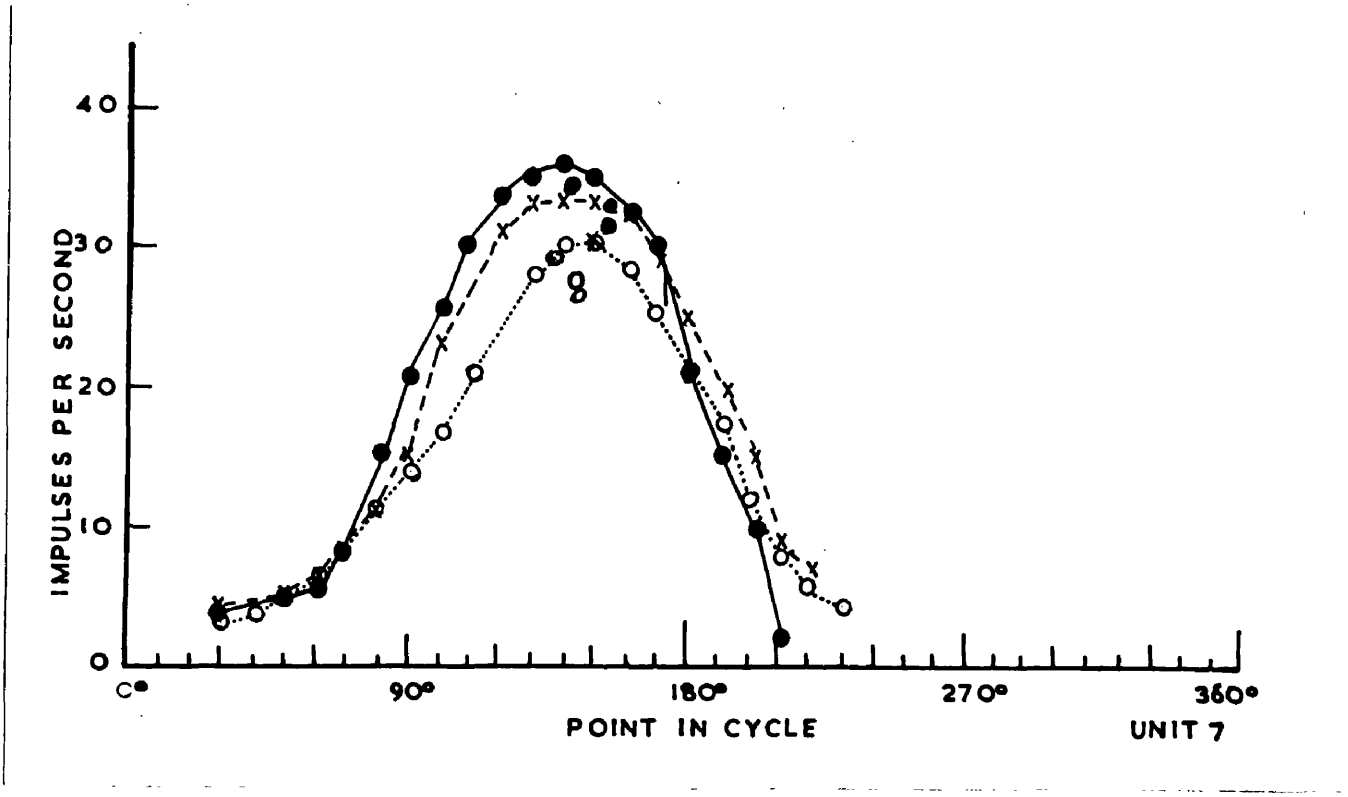

Fig.58. The effect of changes in the value of k_1 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values

o ····· o slow speed
x ----- x medium speed
● —— ● fast speed.

Calculated peak values

		k_1	k_2	k_3
o ●	slow fast	50	15	18
o x ●	slow med. fast	53	15	18
o ●	slow fast	58	15	18

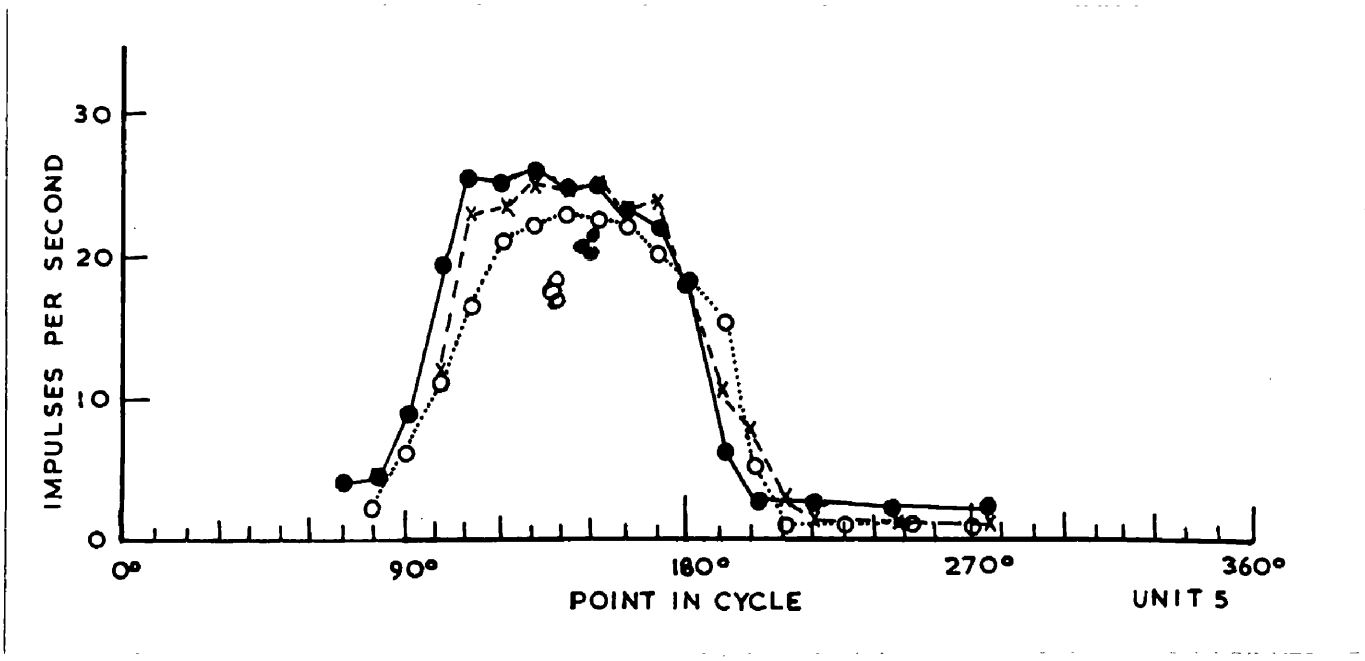

Fig.59. The effect of changes in the value of k_2 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values o ····· o slow speed
× ---- × medium speed
● —— ● fast speed.

Calculated speak values		k_1	k_2	k_3
o slow ● fast		38	7	10
o slow ● fast		38	8.9	10
o slow ● fast		38	10	10

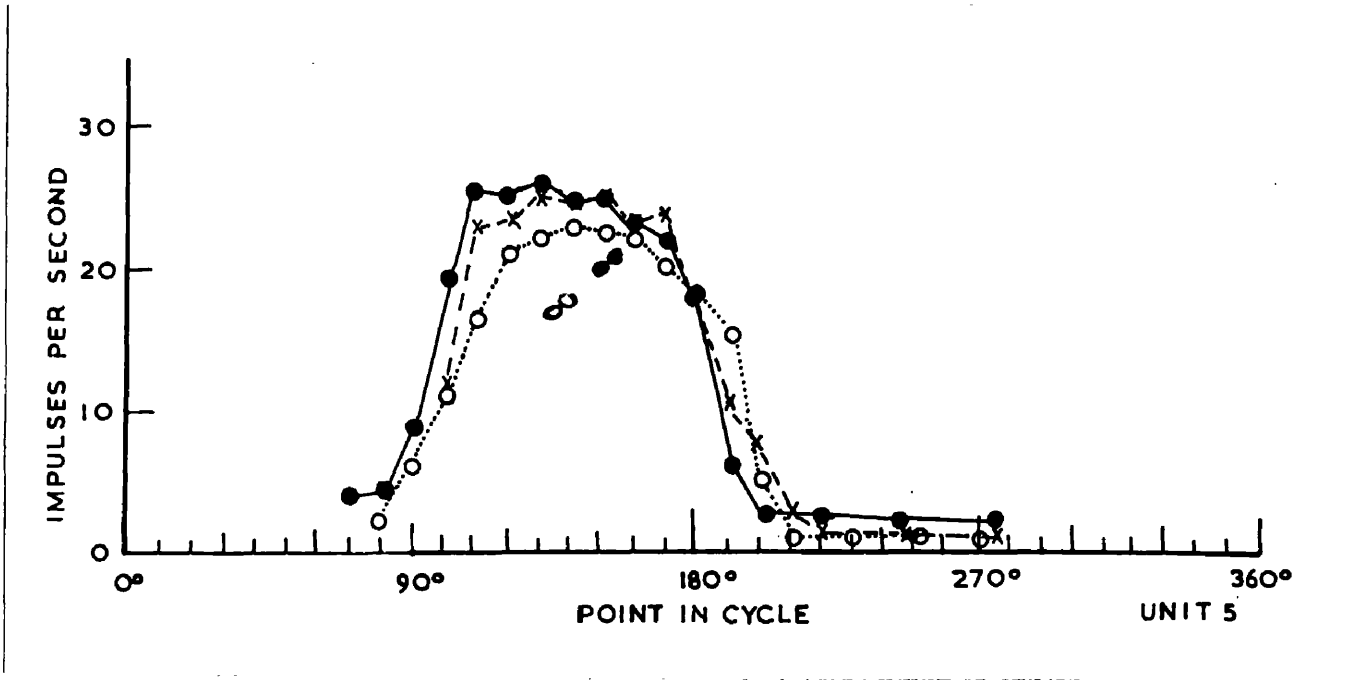

Fig.60. The effect of changes in the value of k_3 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values
o ..... o slow speed
x ---- x medium speed
● —— ● fast speed.

Calculated peak values

		k_1	k_2	k_3
o	slow	38	8.9	10
●	fast			
o	slow	38	8.9	12
●	fast			

Thus the changes in the value of the parameters which might lead to better agreement between the calculated and the observed responses are:-

1) A decrease in the value of the time constants τ_1 and τ_3
2) An increase in k_1 and k_3.

The observed and the calculated responses to the constant velocity steps were then re-examined to see if there would be any justification for making such changes.

On examination of the response curves, it appeared that agreement between the observed and the calculated responses in units 5, 7, 8 would be improved by such changes; the most marked changes being required in unit 5. From inspection of the graphs of the response to the constant velocity steps, in unit 5, new values of the time constants τ_1 and τ_3 were decided upon, and the peaks of the response plotted using the old and new values (Fig.61). The new values improved the position of the peaks but their amplitude, at the two slower speeds, was now too low.

The theoretical response to the sinusoidal movement is composed of two parts:-

a) the velocity component which takes the form A sin Θ + B cos Θ and can thus be represented by a cosine waveform, e.g., C cos $(\theta - \alpha)$,
b) the displacement component, which varies approximately linearly with position and is thus almost sinusoidal in form.

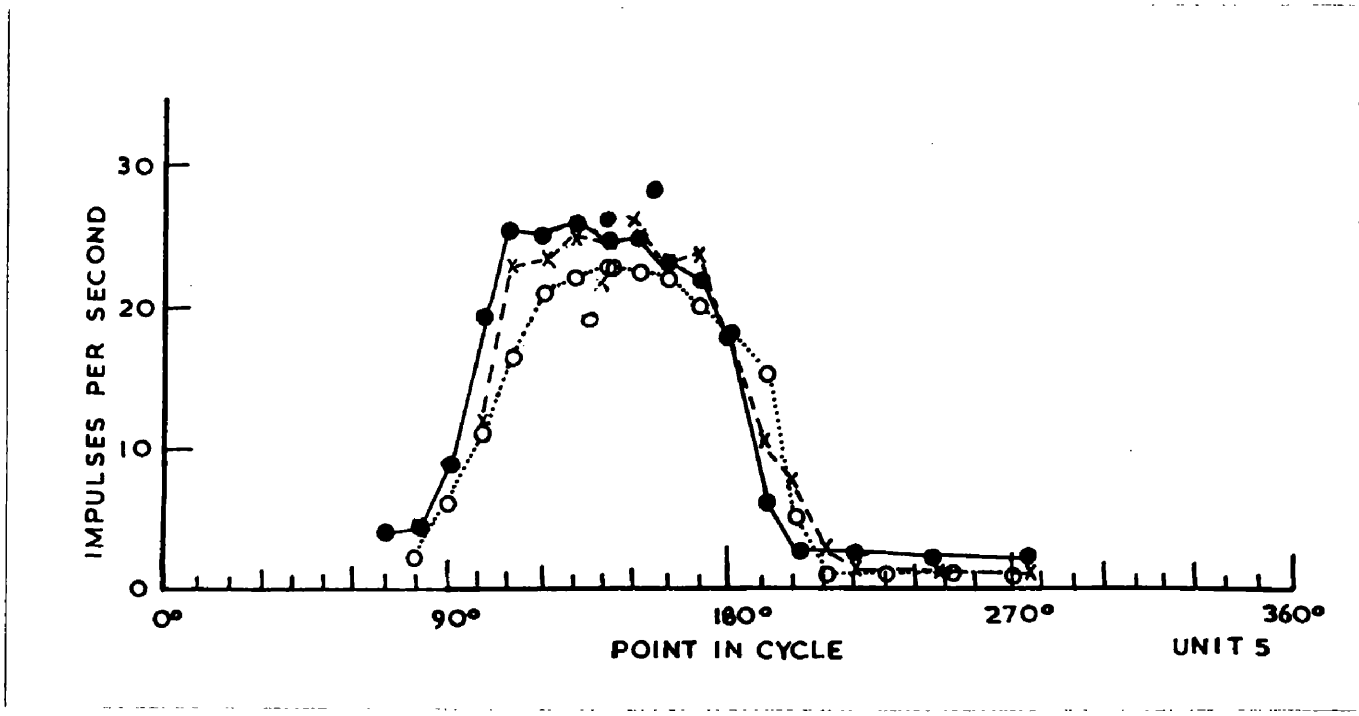

Fig.61. The effect of changes in the value of τ_1 and τ_3 on the magnitude and position of the peak of the calculated response frequency at different speeds.

Observed values O ····· O slow speed
X - - - - - - X medium speed
● ——— ● fast speed.

Calculated peak values		τ_1	τ_2	τ_3
o	slow	.6	1	6
x	medium			
●	fast			
o	slow	.4	1	5
x	medium			
●	fast			

These two components are shown in Fig.62 and their sum in Fig.63.

Thus the theoretical response is nearly sinusoidal in form, with a slight skewness introduced by the displacement component. The shaded areas in Fig.63 represent a negative response frequency and correspond to the periods in the observed response when the ending was silent save for a few random impulses. The receptor was then in a sub-threshold state.

The spindle is unable to fire regularly at a frequency less than 10 impulses/sec. and so, as expected, at points where the theoretical response lies below this value, the observed response was low and irregular.

The observed and the calculated responses to sinusoidal movement, at the fastest speed, are compared in Fig.64. While there is a considerable measure of agreement between the two curves, it can be seen that, during the latter part of the cycle, the observed response falls away much more steeply than the theoretical response. A possible explanation of this finding arises on consideration of the relationship between length and tension for this muscle.

It is known that the relationship between length and tension, for frog's striated muscle, is not completely linear. Buchthal (1942) has constructed length/tension curves for resting striated muscle fibres in the frog, using small

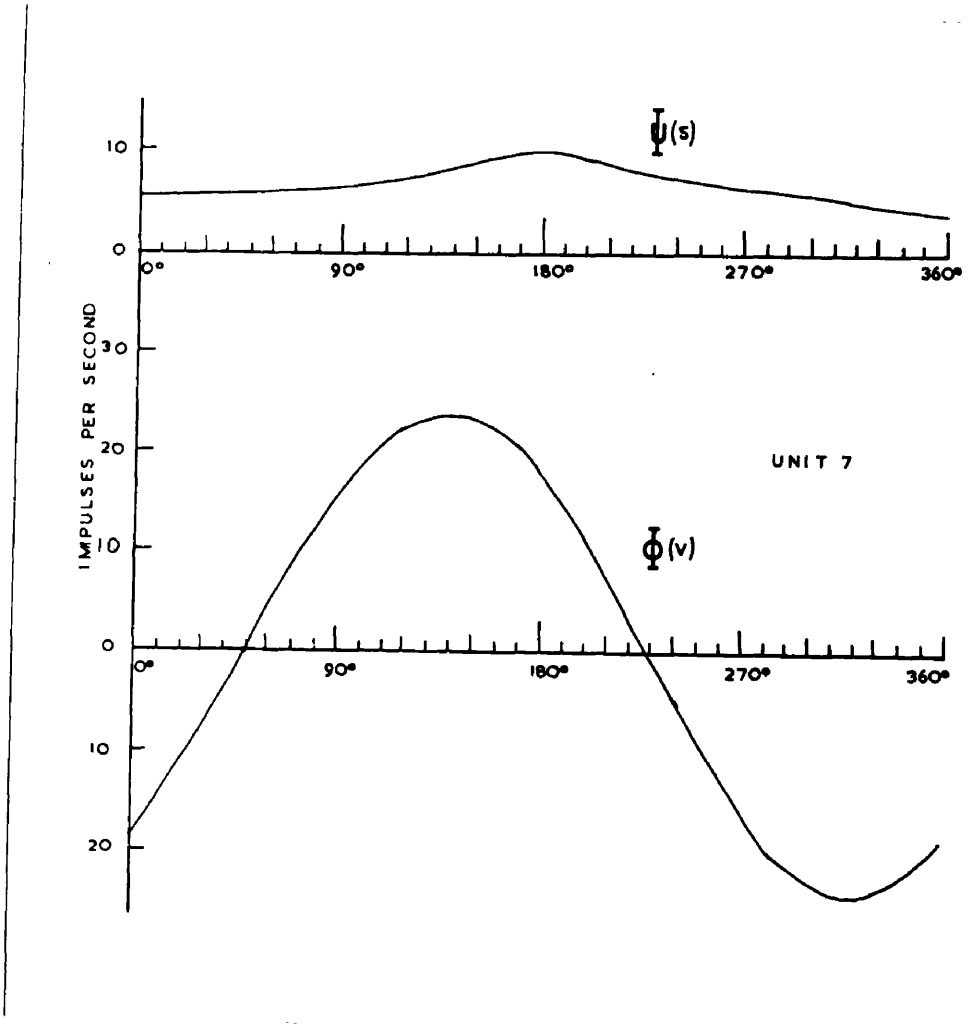

Fig. 62. Graphs showing the two parts of the calculated response frequency due to sinusoidal movement. $\Psi(s)$ the displacement component of the response, $\Phi(v)$ the total velocity component of the response.

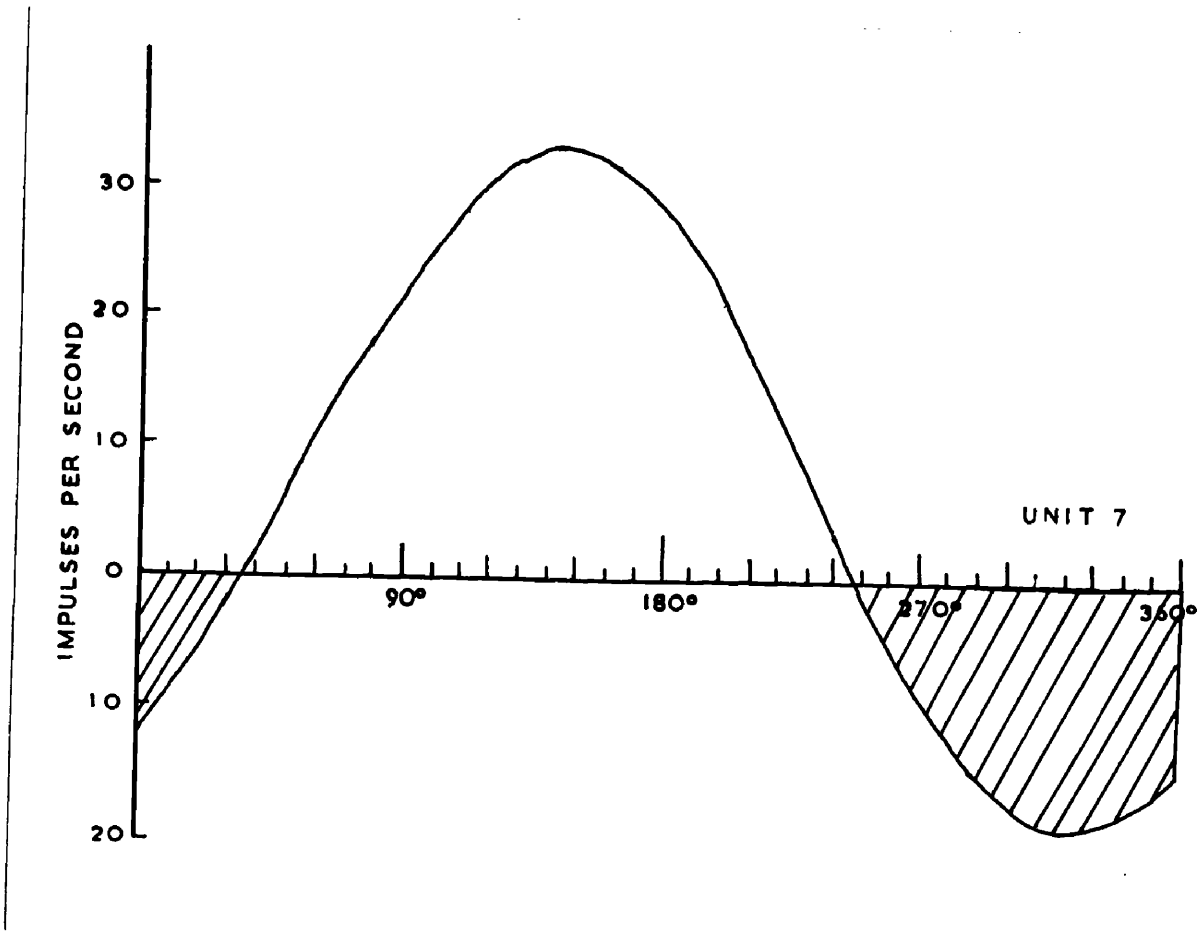

Fig.63. Graph of the calculated total response frequency due to sinusoidal movement — the sum of $\Psi(s)$ and $\Phi(\nu)$ of Fig.62.

The shaded areas represent a negative response frequency and correspond to those parts of the cycle when the ending was in a below-threshold condition.

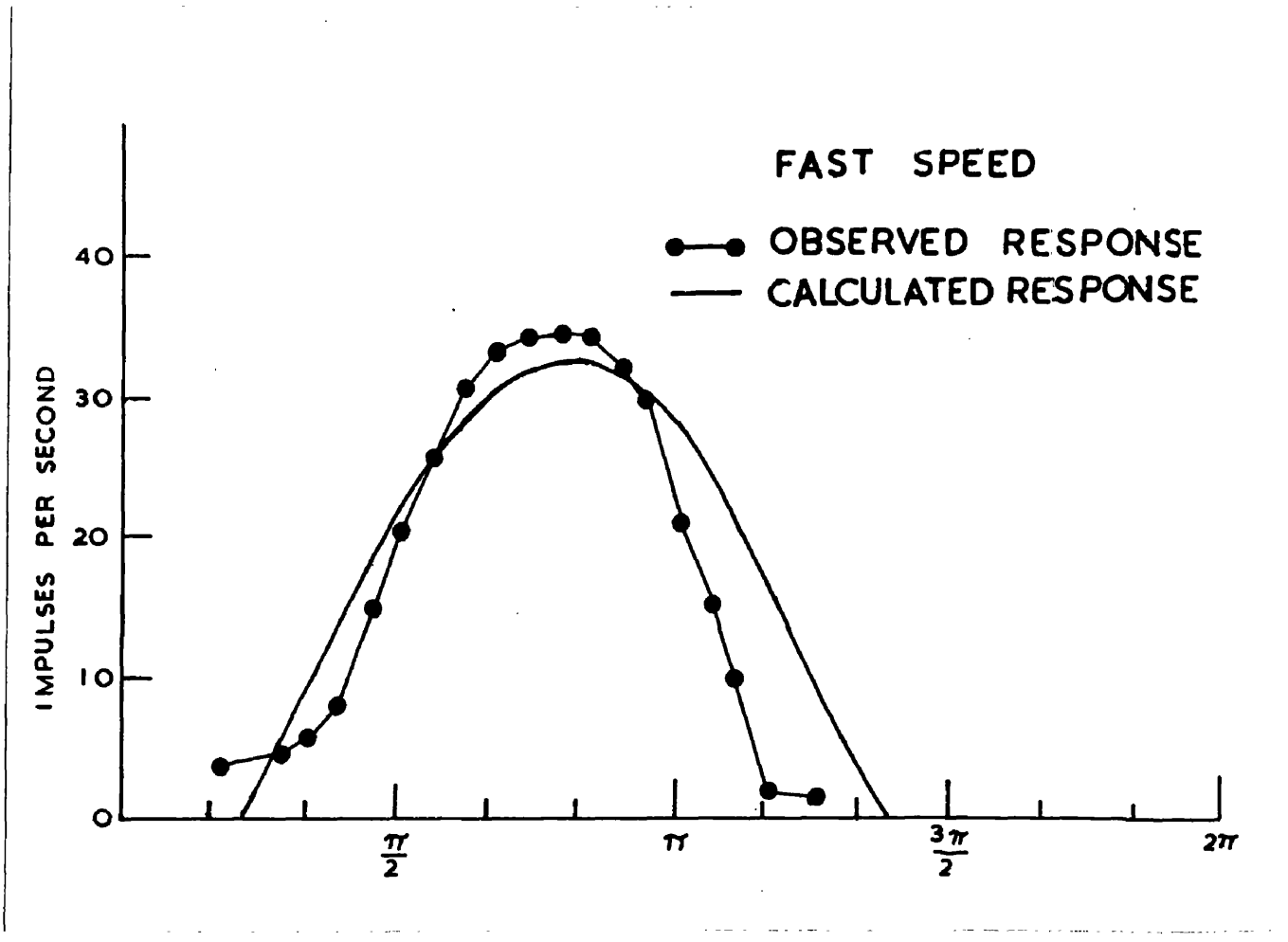

Fig.64. A comparison of the observed and calculated responses to sinusoidal movement at the first speed. The observed response falls away more quickly than the calculated one.

bundles of fibres from the semitendonosus muscle. His results are shown in Fig.65, which shows this relationship determined during movement and under static conditions. He found that, during stretch, the tension, at any length, was greater than the corresponding static tension, and, during relaxation, the tension was less than the stationary value. Buchthal also quotes the same result as found by Blix (1892) for whole muscle. In addition, Blix found that, during cyclical movements, at any particular length, the difference in tension during stretch and relaxation increased with the speed of movement.

It may be that the deformation at the sensory endings is related to the tension in the intrafusal fibres rather than to the extension of these fibres.

During step-like extension of the muscle, over certain ranges, extension and tension are linearly related so that the same results would be obtained irrespective of which was considered as stimulus. During sinusoidal movements this linear relationship no longer holds and if tension is indeed the effective stimulus, the quicker decline of the observed response during the relaxation phase of the sinusoidal movements might be accounted for.

The relationship between length and tension, for this muscle, and the effect on the sensory discharge from the spindle of the application of step-like changes in tension are considered below.

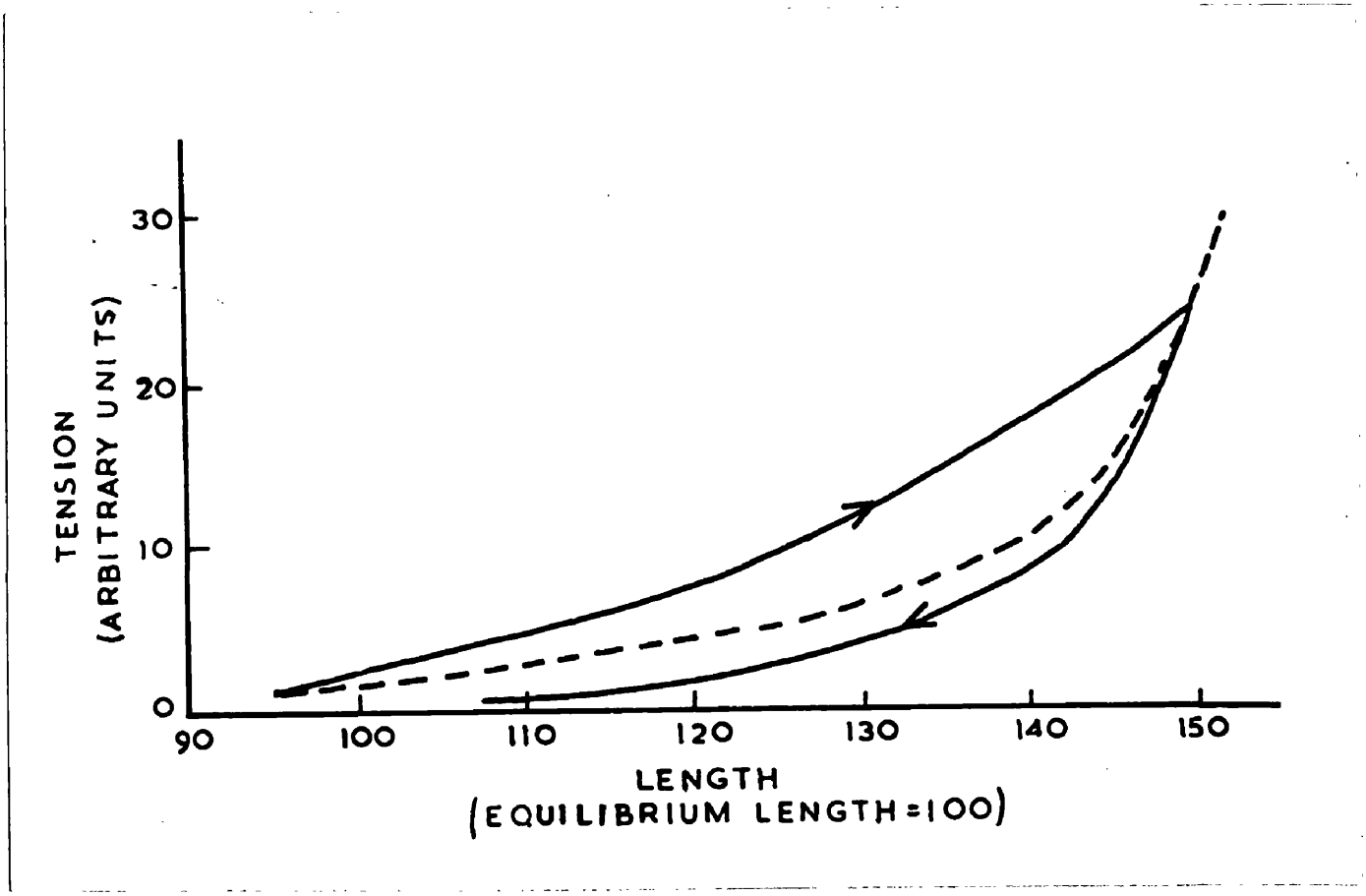

Fig.65. The relationship between length and tension in resting striated muscle fibres of the frog semi-tendonosus determined by Buchthal (1942).

——— represents the relationship found when length and tension were recorded simultaneously during cyclical movement.

..... represents the relationship found when the length of the muscle fibres was measured after the application of tension.

PART 3.

TENSION EXPERIMENTS.

Response to Changes in Tension.

From a comparison of the observed response to sinusoidal movement and the expected response calculated using the transfer function it appeared that the sensory response might be directly related to changes in the tension of the intrafusal fibres rather than to changes in their length. It was therefore felt that it would be valuable to find the effect on the sensory discharge of the application of tension changes similar to the previous length ones.

Some preliminary experiments of this type were done. Step-like increases in tension were applied to the muscle and the sensory response recorded during and after their application.

The apparatus used is described above in Part Ib. 25 experiments were made and on 7 occasions the responses were filmed. The frequency of the responses was plotted as a function of time in 4 experiments, giving a total of 37 steps. The response was analysed in 14 steps from 3 experiments (Table 5).

Before applying the tension steps to the muscle, the tension produced by the moving coil system, with various values of the resistance and voltage of the circuit, was determined by means of the torsion balance. The tensions applied ranged from 0.18 gm. to 1.4 gm. The initial tension of the muscle could not be measured with the transducer used, but was extremely low. Matthews (1931a) reports that the maximum

Table 5.

The number and magnitude of the tension steps applied to each unit, showing the steps in which the response to the application of tension was analysed.

Unit	No.	Tension applied gm.	No. of steps	Response plotted	Response analysed
12	1	0.19	2	+	+
	2	0.23	2	+	+
	3	0.27	2	+	+
	4	0.37	2	+	+
	5	0.39	2	+	+
	6	0.51	2	+	+
	7	0.55	2	+	+
	8	0.64	2	+	+
	9	0.76	2	+	+
14	1	0.18	2	+	+
	2	0.21	2	+	-
	3	0.3	2	+	+
	4	0.4	2	+	+
	5	0.5	2	+	-
	6	0.6	2	+	+
	7	0.7	2	+	Record too short
	8	0.8	2	+	"
	9	0.9	2	+	"
	10	1.0	3	+	"
	11	1.1	1	+	"
15	1	0.24	2	+	Record too short
	2	0.4	2	+	"
	3	0.6	1	+	"
	4	0.8	2	+	"
	5	1.0	2	+	"
16	1	0.18	2	+	+
	2	0.3	2	+	+
	3	0.4	2	+	-
	5	0.6	2	+	-
	6	0.7	2	+	-
	7	0.8	2	+	-
	8	0.9	2	+	-
	9	1.0	2	+	-
	10	1.1	2	+	-
	11	0.9	2	+	-
	12	1.1	2	+	-
	13	1.4	2	+	-

The responses to 37 steps were plotted and measured.
The graphs of the responses to 15 steps were analysed.

tension developed in M. ext. long. dig. III during tetanic stimulation was 10 gm., so that he considers 2 gm. to be about the maximum tension occurring in life. It therefore seems likely that the tensions applied to m. ext. long. dig. IV were all within the physiological range.

After each step had been applied the muscle was kept under this tension for 2 min. The response was filmed for the first 30 sec. after the application of stretch and then for 5 sec. periods at 30 sec. intervals. The tension was then released and 3 min. allowed to elapse before the next pull.

The form of the discharge was similar to that recorded in response to the extension steps except that, in this case, the maximum response frequency was much higher. It was therefore more regular since it was well above the firing threshold. It was found that repeated pulls, at the same tension, gave very similar responses (Fig.66), so, normally, only two pulls were made at each tension.

The analysis of the response was carried out in an exactly parallel manner to the previous analysis of the extension steps of constant velocity. The frequency of the response after 2 minutes under a particular tension was taken as the static component Ψ of the response at that tension. The remainder of the response, total frequency $F - \Psi$, was then analysed into the three 'velocity' components, Φ, ,

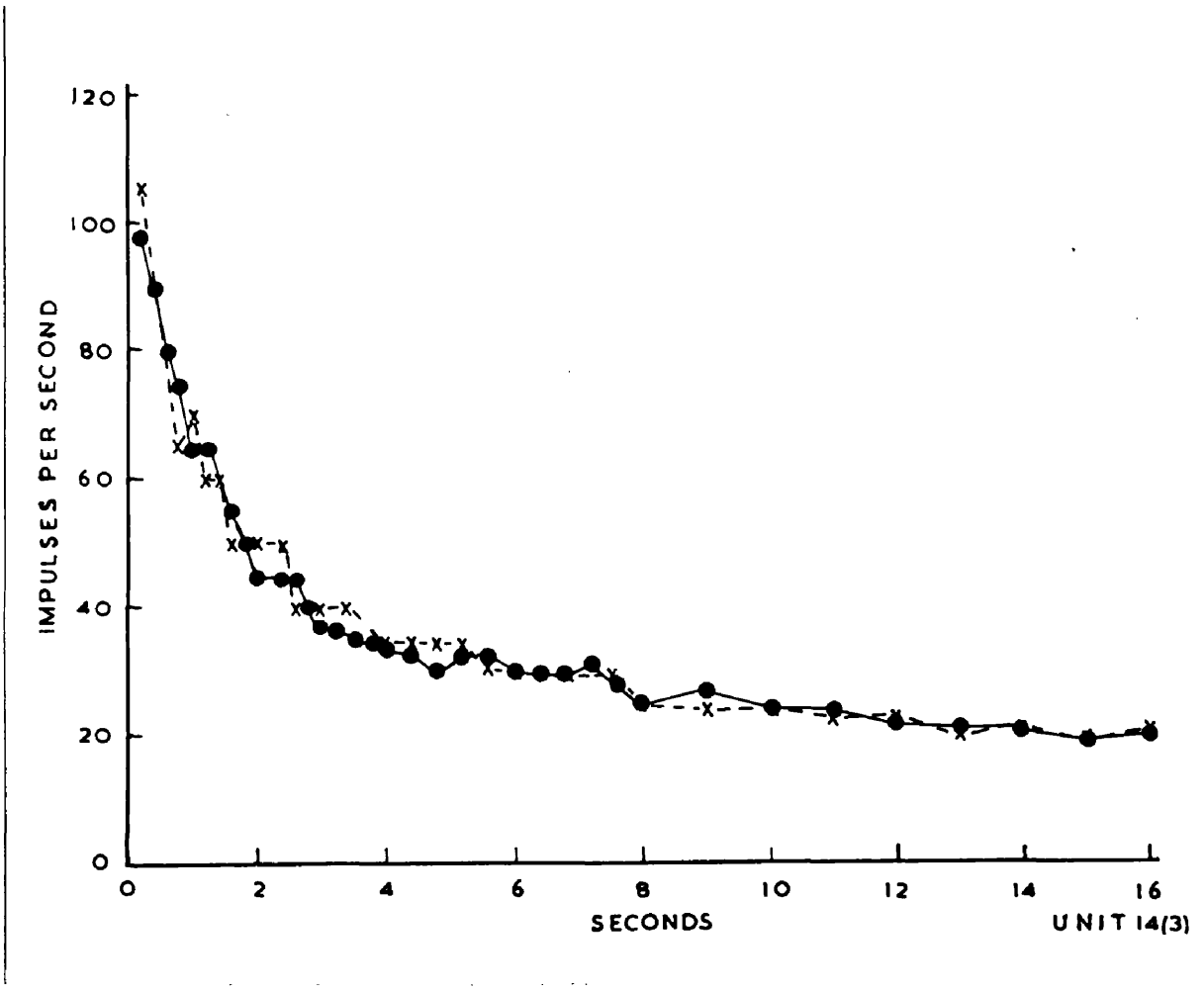

Fig.66. Graphs of the responses to two successive applications of the same tension 'step', showing the repeatability of the response

●——● 1st step

×---× 2nd step.

The muscle was kept under the applied tension for 2 min. This tension was then removed and 3 min. allowed to elapse before it was re-applied.

Φ_2 and Φ_3 as before. (The term 'velocity' is used here to express rate of increase in tension.)

The six parameters $\phi_1, \phi_2, \phi_3, \tau_1, \tau_2 \& \tau_3$ and were evaluated.

ϕ_1, ϕ_2 and ϕ_3 were plotted against tension 'velocity' and the relationship between them was found to be approximately linear, giving $\phi_i = k_i v$ (i = 1, 2, 3) (Figs. 67, 68, 69).

τ_1 and τ_2 were fairly constant both within and between units, while τ_3 shows considerable scatter (Fig.70).

The values obtained for the parameters were compared with the values obtained in the previous analysis of the extension steps ( ). The higher response frequencies recorded here, due to the greater tensions and extensions applied to the muscle, are reflected in higher values of the parameters ϕ_i. However they still bore much the same relationship to each other, i.e. $\phi_2 = \phi_3$, $\phi_1 = 3\phi_2$

The time constants τ_i did not agree with the values obtained for units 5, 7 and 8 of the extension steps but were quite similar to those of unit 9. Unit 9 also resembled the tension steps in that the responses were at a higher frequency than in the other extension steps.

In all these higher frequency responses, about 20 sec. after the new position had been reached the frequency of the

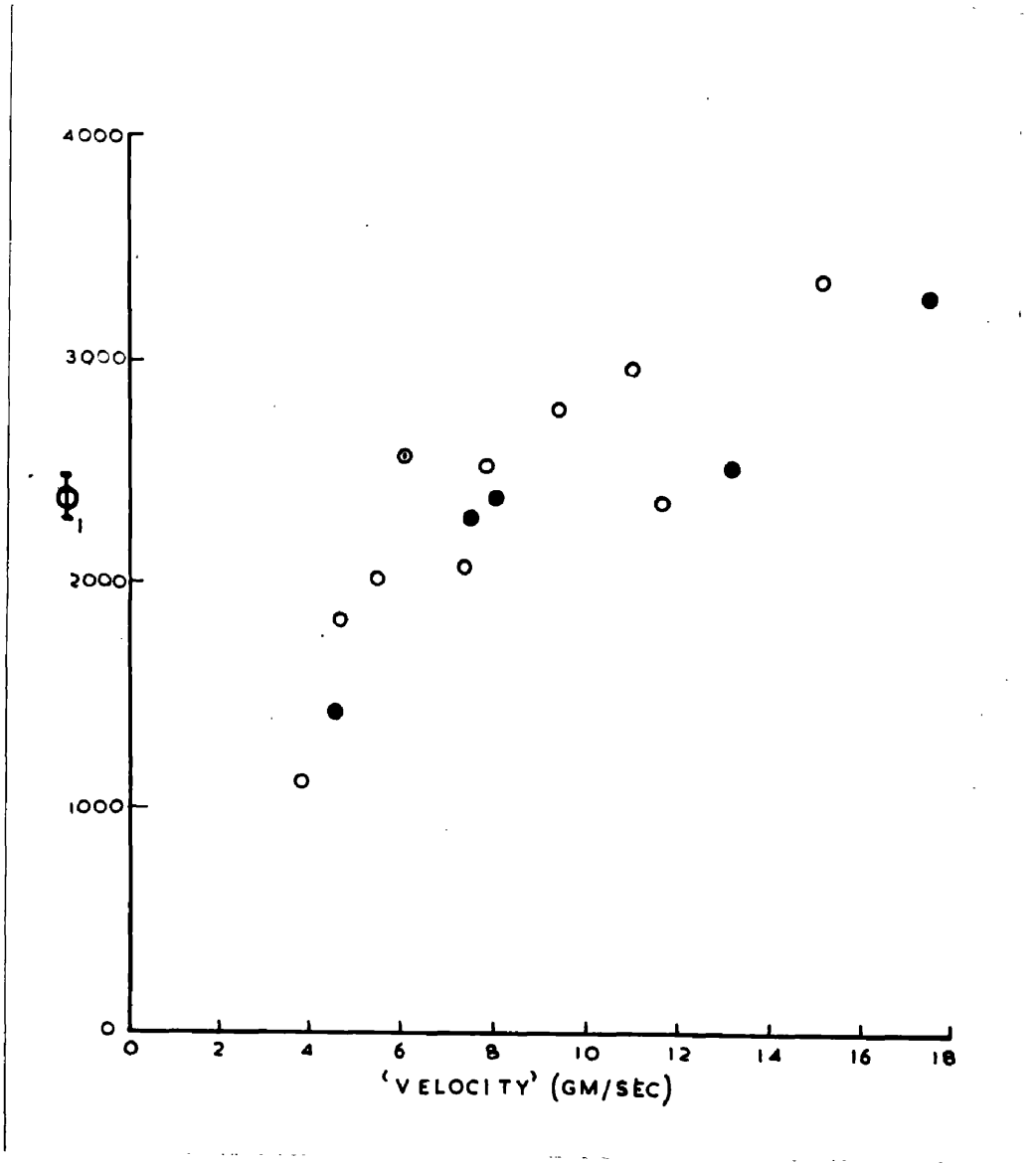

Fig.67. The relationship between the parameter ϕ_1, and the "velocity" (rate of increase of tension) of the tension steps for units 12, 14 and 16.

- o Unit 12
- ● Unit 14
- ⊙ Unit 16.

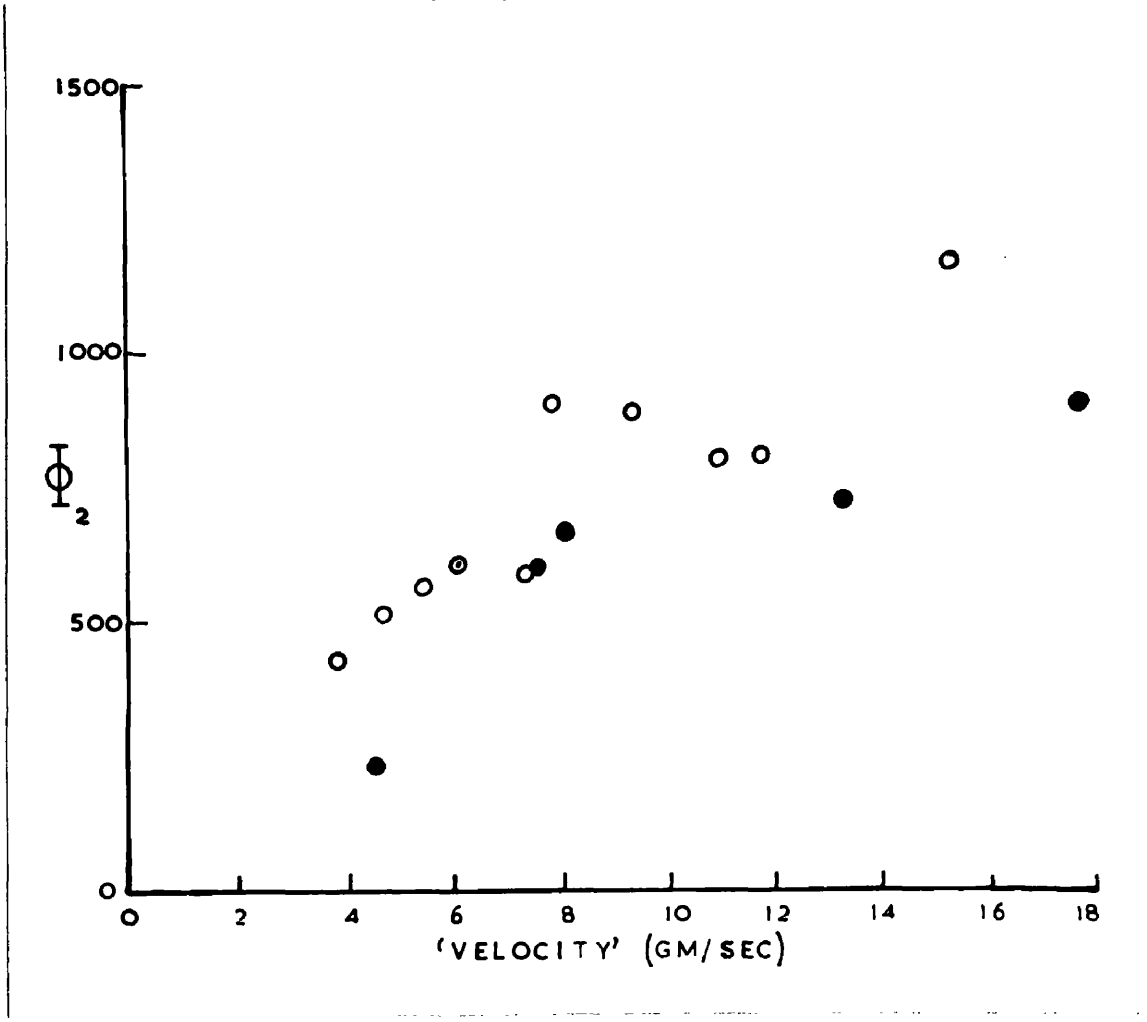

Fig.68. The relationship between the parameter Φ_2, and the "velocity" (rate of increase of tension) of the tension steps for units 12, 14 and 16.

○ Unit 12.

● Unit 14.

⊙ Unit 16.

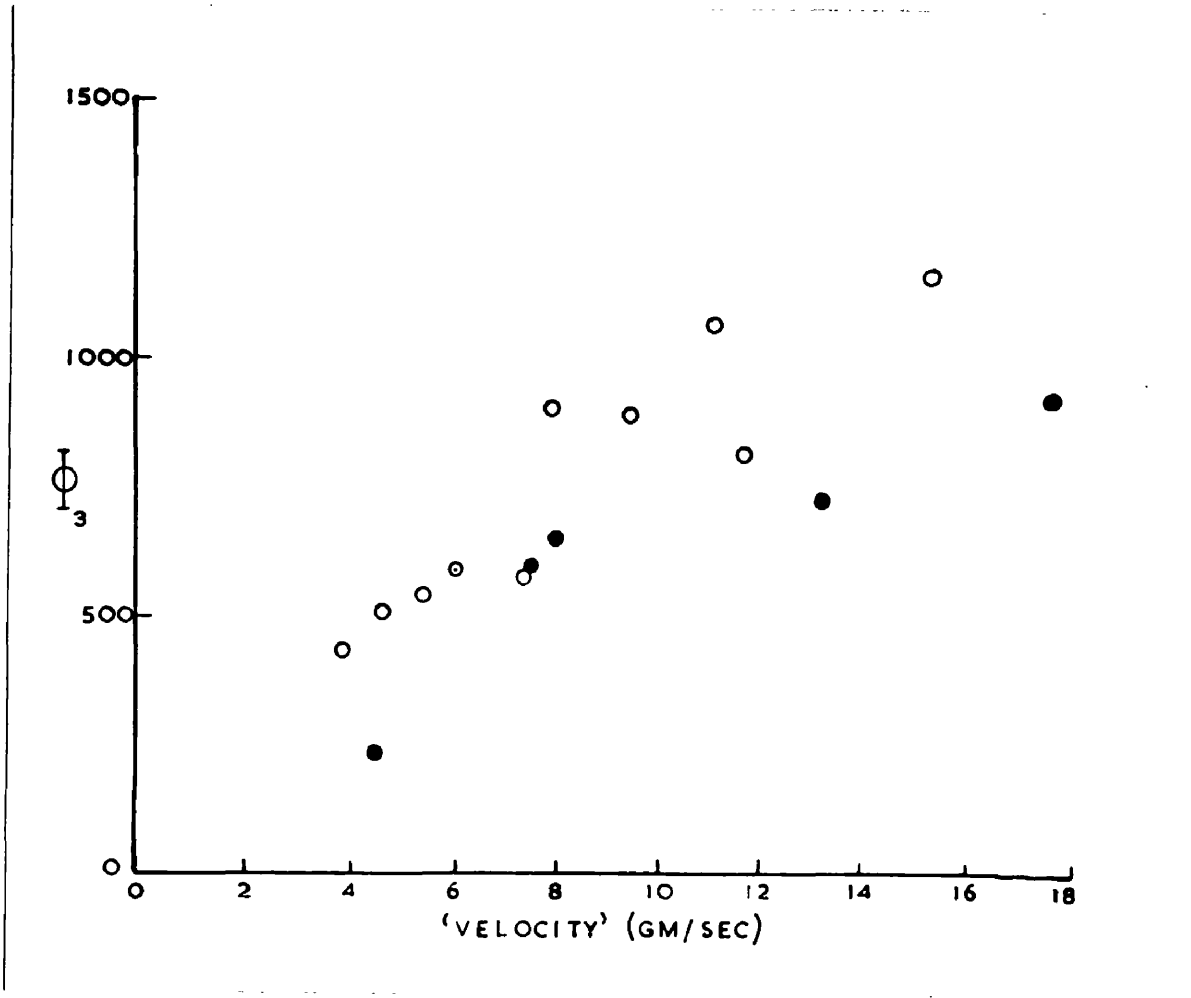

Fig. 69. The relationship between the parameter Φ_3, and the "velocity" (rate of increase of tension) of the tension steps for units 12, 14 and 16.

O Unit 12.

● Unit 14.

⊙ Unit 16.

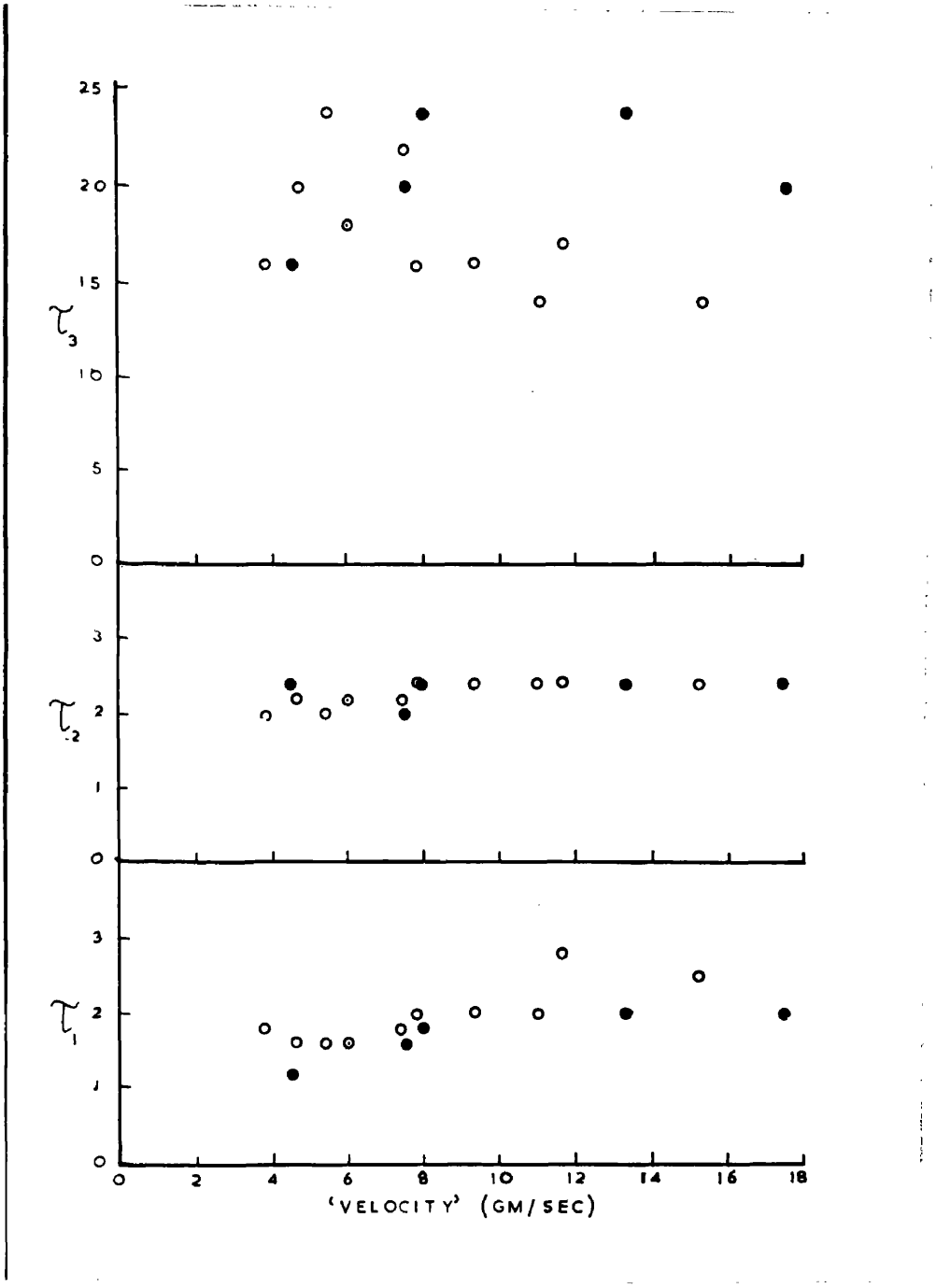

Fig.70. The relationship between the parameters τ_1, τ_2 and τ_3 and the "velocity" (rate of increase of tension) of the tension steps for units 12, 14 and 16.

- O Unit 12.
- ● Unit 14.
- ⊙ Unit 16.

discharge had adapted to practically the same value irrespective of the velocity of the movement by which this position was reached. Therefore it would appear that the frequency of the response at this point is independent of velocity, and could be taken as due solely to position, i.e., the displacement component Ψ. The value which was in fact chosen for Ψ was the adapted frequency 2 min. after the movement, which is lower than the 20 sec. value. It may be that there is a very slow adaptation of the displacement component. If this is so, the use of the 2 min. values for Ψ will affect the determination of τ_3 leading to a much larger value than the true one. In units 5, 7, 8, the frequency had adapted to below 10 imp./sec. in 20 sec. Thus any slow adaptation effect was not noticeable and would not affect the analysis.

An estimate was made of the values of τ_3 which would be obtained for the tension steps, if the 20 sec. value of the response was taken as Ψ. The values obtained were much lower and more consistent: the old values of ranged from 14 to 24 sec., the new values were 5 to 6 sec.

There were several additional sources of error in this analysis to those encountered in the previous one.

a) Measurement of Duration of Movement. The period during which tension was applied was very short, therefore it was difficult to measure it very accurately. Also, although the rate of application of tension was reasonably linear, in some experiments there was an increase in the spindle response before the tension increase was signalled. This was probably due to the muscle being originally in a relaxed state.

From the considerations of the analysis of the constant velocity steps above, it appears unlikely that errors in the measurement of the duration of the movement will have much effect on the parameters derived from the analysis.

b) Measurement of Peak Frequency. On application of the higher tensions, the maximum frequency of the response was very high; little more than the refractory period of the nerve separated the impulses. On some occasions, the film speed was not fast enough to make the impulses at the peak of the response easily separable. Since the film speed could not be altered during filming, this meant that if the speed was fast enough for the impulses at the peak of the response to be clearly distinguishable then the film was liable to run out before a sufficient length of record for analysis had been obtained. There would therefore sometimes be an error in the measurement of the peak frequency. In these

cases, some point on the response curve, other than the peak, was used in the calculation of the parameters ϕ_1 and ϕ_2.

In Fig.71, the observed and the calculated responses to a tension step are compared. A good fit of the experimental curve was obtained for all the steps. While there appears to be a slight improvement in the fit of the observed responses, it is not significantly better than that obtained for the extension steps. This is the expected result since, during step-like extensions of the muscle over certain ranges, the relationship between tension and extension is almost linear.

Since these tension steps were not accompanied by corresponding sinusoidal changes in tension, they give little additional evidence for the view that tension, rather than extension, was the effective stimulus to the spindle. They do, however, give further support to the idea of the applicability of this transfer function to the frog neuromuscular spindle.

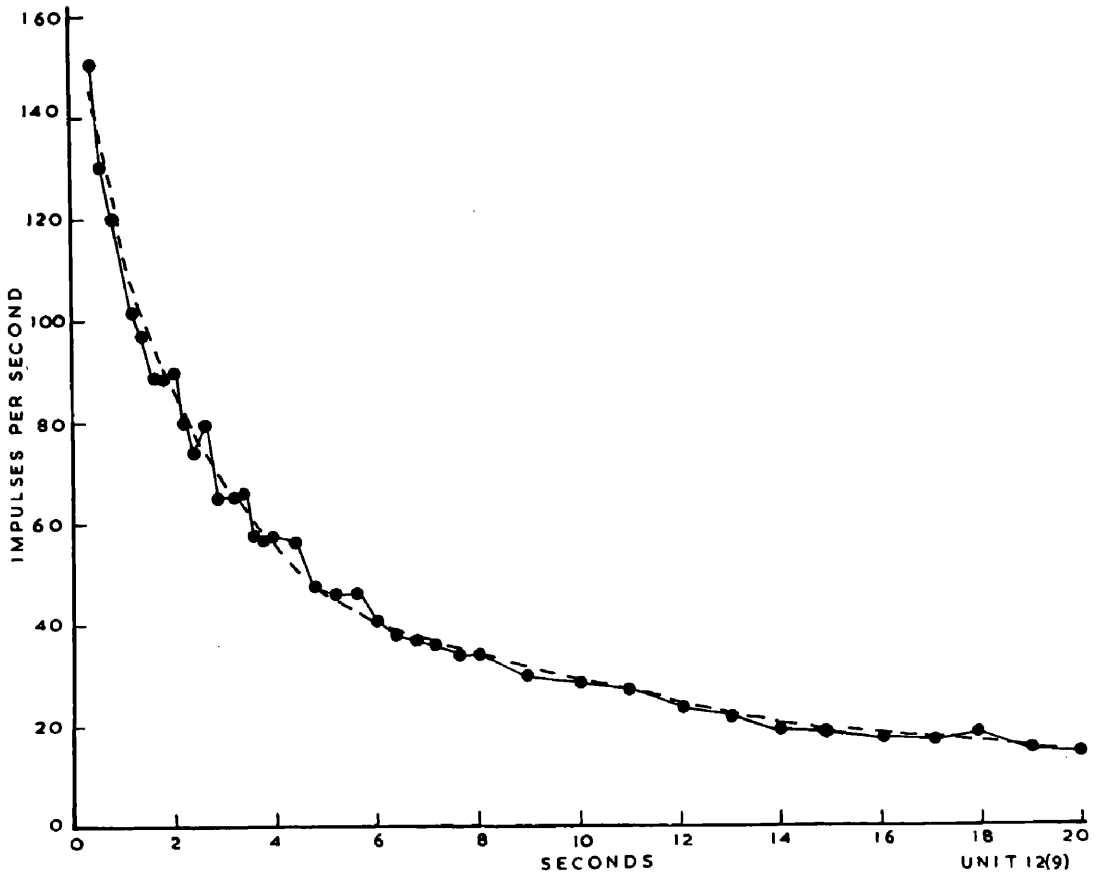

Fig.71. A comparison of the graphs of the observed and the calculated responses to the application of a tension "step" (unit 12(9)).

● ——— ● observed response,

– – – – calculated response.

Length/Tension Relationship.

It has been shown that the responses to steps of both extension and tension can be fitted satisfactorily by analysis on the basis of the transfer function derived from the cat knee joint proprioceptor. However when the parameters derived for the constant velocity steps are used to calculate the expected response to sinusoidal variations in position, the agreement with the observed result is not as close as would be desired.

From a consideration of the relationship, determined by Buchthal (1942), between length and tension for frog striated muscle fibres, the idea arose that deformation at the sensory receptors might be related to the tension in the intrafusal muscle fibres, rather than to the extension of these fibres. It would therefore be worth determining the relationship between length and tension for this muscle over the length range used in the extension experiments.

In many muscles it would be unjustifiable to assume that the tension changes within the spindle followed a similar course to those of the whole muscle. The structure of this muscle, however, makes it appear possible that, in this case, events within the spindle do mimic those of the muscle as a whole. The m. ext. long. dig. IV is a thin strap-like muscle about 16 mm. long and the spindle intrafusal muscle fibres run

parallel to the extrafusal ones and from end to end of the muscle. Thus experiments on the whole muscle may give information about events within the spindle.

Using the apparatus described above, sinusoidal variations in tension were applied to the muscle by varying the current passing through the moving coil system. The length and tension of the muscle were measured simultaneously during the movement.

These measurements were carried out on 7 muscles.

The resting length of the muscle in situ was measured and the initial length was set to approximately this value. The current passing through the coil was adjusted so that the maximum extension of the muscle was about 3 mm., i.e., the muscle was being stretched over the same length as was used for the constant velocity steps.

Length and tension were recorded simultaneously on calibrated paper on the pen recorder. Five cycles were recorded at each of the speeds used. The same three speeds of sinusoidal movement were used as in the sinusoidal length variations, i.e., 0.2, 0.3 and 0.4 cycles/sec.

Fig.72 shows the form of the relationship and the effect on it of change in the speed of the movement. At any length of the muscle, the difference between tension during extension and during relaxation was always found to increase with speed to a greater or less degree. Thus if the deformation of the

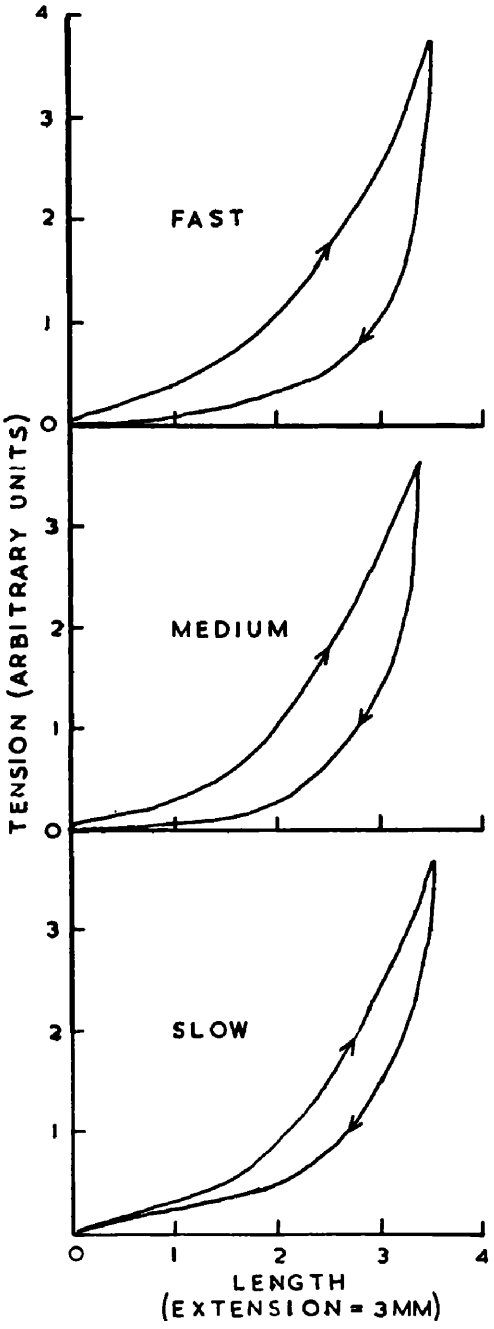

Fig.72. The relationship between length and tension for m. ext. long. dig. IV, determined during sinusoidal movements at three different rates:

fast (0.4 cycles/sec.),

medium (0.3 cycles/sec.),

slow (0.2 cycles/sec.).

receptors is directly related to the tension of the intrafusal fibres then it would be expected that the agreement between the observed and the calculated responses to sinusoidal extensions would be worst at the fastest speed. That is what had been already found to be true.

A comparison between the observed and the calculated responses to sinusoidal movement at the fast speed was shown in Fig.64. The expected response was calculated on the assumption that the sensory discharge was directly related to the length changes in the muscle. Suppose, now, that the observed response is, in fact, directly related to the tension changes in the muscle, then, by correlating, for particular times, the observed response (supposed to be related to tension) with the expected response (calculated on the basis of extension) we can obtain a relation between tension and extension. This has been done for sinusoidal movement at the fast speed and is shown in Fig.73. The relationship between length and tension obtained in this way is similar in form to those determined directly and shown in Fig.72.

Since the recording of the length/tension relationship, and of the sensory responses to sinusoidal length changes, have not so far been carried out, both on the same muscle, no direct comparisons are possible. However, since the experimental conditions were made as nearly similar as

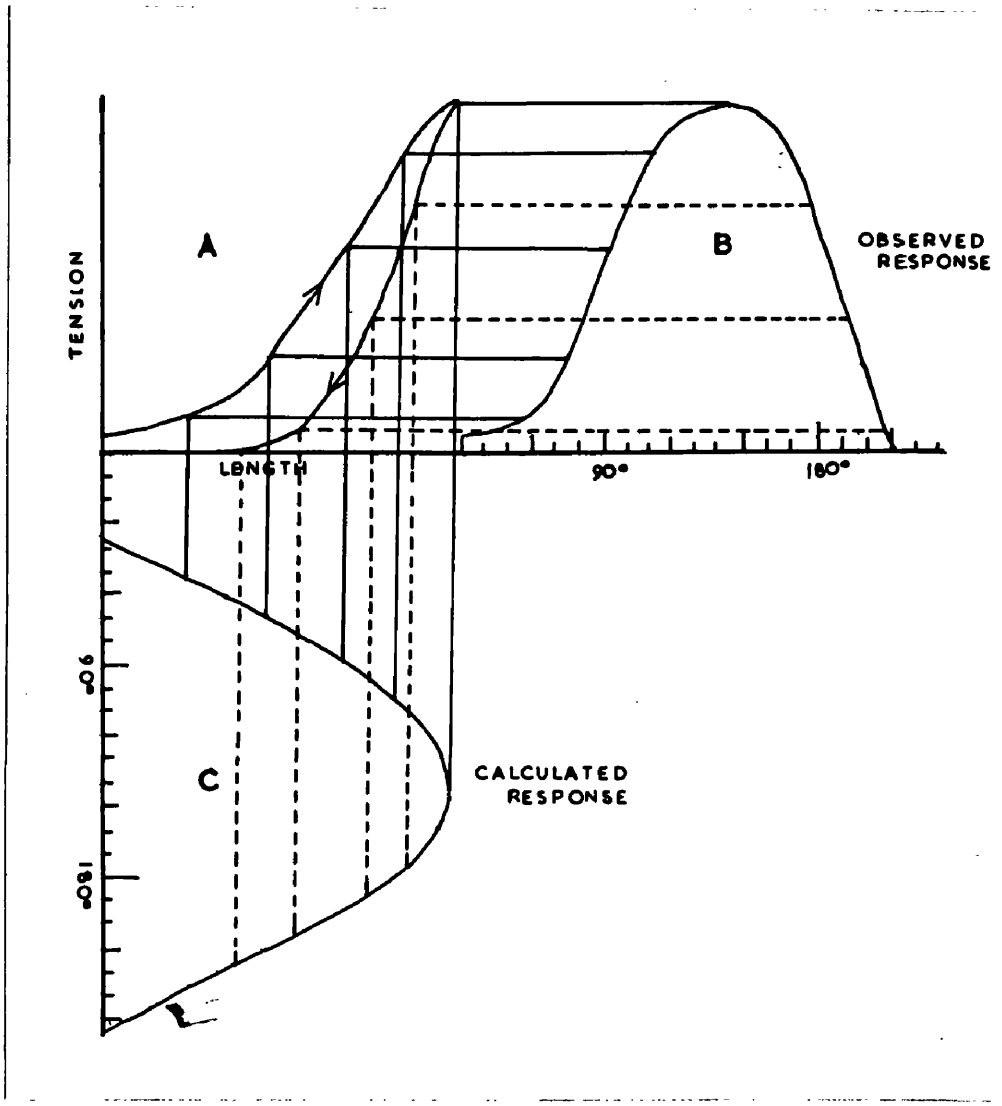

Fig.73. The relationship between length and tension of m. ext. dig. IV, during sinusoidal movement at the fast speed. (A) which is obtained if it is assumed that the actual discharge frequency recorded in the sensory nerve during the movement (B) is directly related to tension changes within the muscle. The expected response (C) was calculated on the assumption that it was directly related to length changes in the muscle. By correlating graphs B and C at various times throughout the movement, the relationship A is obtained.

possible, it seems valid to assume that the same type of tension changes, in response to sinusoidal movement, would take place in the muscles during the experiments described in Part 2, as in the length/tension determinations.

If changes in tension were the effective stimulus to the spindle, then, during sinusoidal changes in position, the input to the spindle would no longer be sinusoidal, and this would perhaps explain the quicker decline in the observed, as compared with the calculated, response, during the relaxation phase of the movement.

Discussion.

The responses of the frog neuromuscular spindle to known stretches were analysed to determine the form of the relationship between the input and output of the spindle, i.e., the transfer function, and to see whether this transfer function had the same form as that determined for the cat knee-joint proprioceptor.

Before determining the form of this transfer function it must be shown that the spindle gives consistent responses under standard conditions.

In the experiments reported in Part 2 of this thesis, some features of the experimental conditions, which may have affected the response, were not rigorously controlled. Variations in these conditions might be held to be responsible for certain variations in the response. For example, the preparation remained in the same Ringer solution throughout the experiment, after having been placed in fresh Ringer when the dissection was complete. According to Matthews (1931a) this would perhaps constitute a source of error and lead to a diminished response to a particular stimulus, possibly due to an accumulation of potassium ions.

The information available about Ringer effects from the present experiments is as follows. a) On one occasion the preparation was left overnight and on the following morning gave about 70% of its previous response. b) On

several occasions the same stretch was repeated at times from one to three hours apart. It was found that over 2-hour intervals the response was little altered.

Thus, from the evidence available in these experiments it appears that the sensory discharge was little affected by keeping the muscle in the same Ringer throughout. In Matthews' experiments the muscle was being stretched more vigorously and more often, so that adverse Ringer effects might be more pronounced.

The time chosen for the interval between stretches was 3 min. From Fig.24 it can be seen that this interval was not always long enough to allow for the full return of excitability of the spindle, since if a greater interval was allowed, the response increased. On the basis of Bronk's (1929) work on the effect of repeated loading on the discharge from a stretch receptor Matthews (1931a) recommends that a period of 5 min. should be allowed for the full return of excitability of the spindle in m. ext. br. prof. dig. III. The reason for choosing 3 min. as the interval allowed between successive stretches was the time involved in the experiments. If the whole experiment was to be carried out as planned, using 3 min. pauses, then a minimum of 5 hours' recording time would be involved. In the early experiments several hours were taken to obtain a single unit response from the muscle.

Despite these factors, which may have introduced uncontrolled variations in the response, it appears from Fig.23 that, over short intervals of time, the response to stretch was very consistent. This preparation is therefore a suitable one to use for the mathematical analysis necessary for the derivation of a transfer function.

The transfer function links the impulse frequency recorded in the afferent nerve with the stimulating movement applied to the spindle. Katz (1950b) has shown that the impulse frequency in the sensory nerve from the spindle in m. ext. long. dig. IV is directly related to the depolarisation at the sensory terminals. He plotted the impulse frequency against the depolarisation level and fitted a regression line to the graph. He found the slope of this line to be highly significant. The transfer function is thus, in effect, linking the depolarisation at the sensory terminals with the stimulating movement.

Katz divided the depolarisation at the sensory terminals into two parts - a static component and a dynamic component. The static component showed an approximately linear increase with the amplitude of the stretch. The dynamic component increased with the rate of stretching. Katz' tentative hypothesis is that the dynamic component may be due to a change in membrane capacity and the static component to a change in membrane permeability.

The transfer function derived for the knee-joint proprioceptor of the cat is composed of two parts - a part due to the position, s, of the joint - the displacement component $\Psi(s)$ and a part due to some memory of the velocity, v, by which this position was reached - the velocity component $\Phi(v)$

The response of the cat knee-joint proprioceptor and of the frog neuromuscular spindle to similar types of constant velocity steps are compared in Fig.28. The general form of the spindle response resembles that of the knee-joint proprioceptor. After the muscle has been stretched the frequency of the spindle response adapts finally to a steady value in the new position. From Fig.25 it can be seen that for any position the same final value is attained irrespective of the velocity with which the alteration in length took place. This final steady value is taken as being due solely to the position of the muscle, i.e., it is the displacement component Ψ of the spindle response. Fig.26 shows that the final adapted frequency in various positions varies linearly with position. Thus the displacement component of the spindle response has the same form as that of the knee-joint proprioceptor. This displacement component may be related to Katz' static component of the depolarisation at sensory terminals which also varies linearly with position.

For the knee-joint proprioceptor the form chosen for the memory of velocity, i.e., the velocity component of the transfer function was an exponential function. This was based on Katz' expression for the depolarisation produced by the capacity changes which occurred on stretching a charged membrane (the dynamic component of the depolarisation). To obtain a good fit of the experimental response it was necessary to postulate three velocity components one negative. It was found that the spindle response could also be fitted by this form of velocity component.

From the analysis of the response of the spindle to stretches of constant velocity (Figs.30-34) it appears that a similar form of transfer function to that applicable to the knee-joint response gave a good fit of the experimental results. However, on using the parameters from the steps to calculate the expected response to sinusoidal movement, the fit obtained was not so good.

A consideration of the relationship between length and tension for this muscle during sinusoidal movement (Fig.72) suggests that the discrepancy between the observed and the calculated responses may be due to the fact that the deformation at the sensory terminals (which is responsible for the depolarisation) is directly related to the tension in the whole muscle rather than to the extension of the muscle.

It seems that a similar form of transfer function to that derived for the cat knee-joint proprioceptor can be used to represent the stimulus-response relationship of the frog neuromuscular spindle, but that, in this receptor, the deformation at the sensory terminals appears to be related to a change in tension of the muscle rather than in extension.

The form of the transfer function thus appears to represent some aspect of the fundamental process involved in the initiation of sensory impulses at the receptor.

PART 4.

HISTOLOGICAL STRUCTURE OF THE FROG NEUROMUSCULAR SPINDLE.

Structure.

Review of literature.

The first description of the frog muscle spindle was given by Weismann in 1861. He described it as a group of fine muscle fibres, of variable diameter and numbering 6, 8, 10 or more: these fibres were of equal length, stretching from tendon to tendon, and bound together by a cord around the middle of their course. They appeared to be covered by a dull granular substance.

In the following years a number of workers added to the knowledge of spindle structure and speculated as to its function. After various ideas, such as growth centre and regeneration area of muscle had been put forward, the view that the frog spindle was a sensory receptor in the muscle gained general acceptance (especially after Sherrington's proof of the sensory nature of the mammalian spindle in 1894).

From this earlier work the following picture of the structure of the frog neuromuscular spindle can be built up.

The spindle is an encapsulated end organ containing a number of thin muscle fibres - intrafusal muscle fibres. Among these muscle fibres are nerve endings.

Intrafusal muscle fibres.

There are from 3 to 7 of these fibres with a diameter of 3-15 μ (Kolliker, 1862). Each fibre shows cross-

striations and has a collection of nuclei in the mid axial region, where the striations are lost.

Sensory ending.

A large diameter medullated nerve fibre enters the spindle in the nucleated region (Cajal, 1888, Hines, 1930, Wilkinson, 1929). This fibre divides up into a large number of very fine filaments which lie parallel to the intrafusal muscle fibres. Many descriptions of this ending have been given. These all seem to agree with Huber and De Witt's (1897) description of "fine fibres richly beset with large varicose enlargements".

Motor endings.

Cajal states that there are nerve endings in the striated regions of the intrafusal muscle fibres at a good distance from the capsular swellings. These endings resemble ordinary motor endings except that they are smaller and have fewer end branches. They are found at one or both poles.

Staining technique.

In order to obtain a clearer picture of the structure of the neuromuscular spindle in m. ext. long. dig. IV, the muscle was stained with gold chloride and teased in glycerine. The lay-out of the spindle and the form of the nerve endings present could thus be shown. 30 muscles were stained in this way.

In addition 2 muscles were stained with haemalum and eosin and serially sectioned throughout their length. Examination of these serial sections gave information about the intrafusal muscle fibres and the position of the sensory endings.

Gairns' (1930) modification of the gold chloride method was used and the procedure adopted was as follows:

1. The muscle was put in a solution of 1 part formic acid (S.G. 1.22) to 3 parts freshly filtered lemon juice and placed in absolute darkness for 10 min. At the end of this period, the muscle was removed from the solution and pressed between filter paper to remove excess fluid.
2. The muscle was then placed in a 1% solution of gold chloride and again left in the dark for 10 minutes.
3. After again removing excess fluid from the muscle it was placed in a 25% solution of formic acid and kept in absolute darkness for 24 hours.
4. At the end of this 24 hr. period, the excess fluid was

again removed, the muscle placed in pure glycerine and left in full daylight for several days.

Initially the muscle was dissected out completely from the foot and the staining carried out in a watch glass. A few extra pieces of tissue were added to the fluid in the watch glass to prevent overstaining of the muscle. It was found that this method led to a considerable shrinkage of the muscle fibres. Before staining the length of the muscle was about 15 mm.; after staining the muscle measured 10-11 mm.

It was thus unsatisfactory to stain the muscle when it was completely removed from the foot. Therefore the following procedure was adopted. The muscle was exposed in the usual way and freed from the surrounding tissue, but the attachments of the tendons were left intact. The phalanges of toes I, II, III and V were removed. The strip of tissue and bone which remained was then tied on to a glass rod. Staining was carried out in test tubes of a size just large enough to accommodate the glass rod and the tissue. Thus there was not a great deal of extra staining solution present and over-impregnation of the tissue could be avoided.

After standing in daylight for several days the muscle was detached from the rest of the foot and placed on a microscope slide with a little glycerine for teasing. This was carried out under a Zeiss binocular dissecting microscope

using fine needles and forceps. The extrafusal muscle fibres were removed, taking care to avoid damage to the intrafusal muscle fibres or their innervation. The position of the intrafusal muscle fibres was usually found by following the main nerve trunk into the muscle. When the extrafusal muscle fibres had been removed and the lay-out of the spindle could be seen, a cover slip was placed over it and the more detailed structure examined under the histological microscope. The results of this examination are described below.

Results.

In the 30 muscles examined, the general lay-out of the spindle system varied considerably: no two muscles, even from opposite feet of the same frog, showed identical patterns of innervation.

In all cases the spindle intrafusal muscle fibres ran parallel to the extrafusal ones and from end to end of the muscle. The number of intrafusal muscle fibres found was from 8 to 18.

A photograph of a spindle system is shown in Fig.74. This shows certain features which were common to all the muscles examined.

The intrafusal muscle fibres did not run together throughout their length, but in several bundles of 2 to 6 muscle fibres. These bundles rarely kept separate throughout the entire length of the spindle system, e.g., in Fig. 74 bundles 3 and 4 at end A have united to form a single bundle of fibres at end B. (The arrangement of the intrafusal muscle fibres into bundles is not a dissection artefact. This finding is confirmed by the study of cross sections of the muscle reported below.)

Each bundle of fibres has at least one sensory region and often there are several such regions on one bundle, e.g., bundle 3 of Fig.74 has 3 sensory regions f, g and h. Often

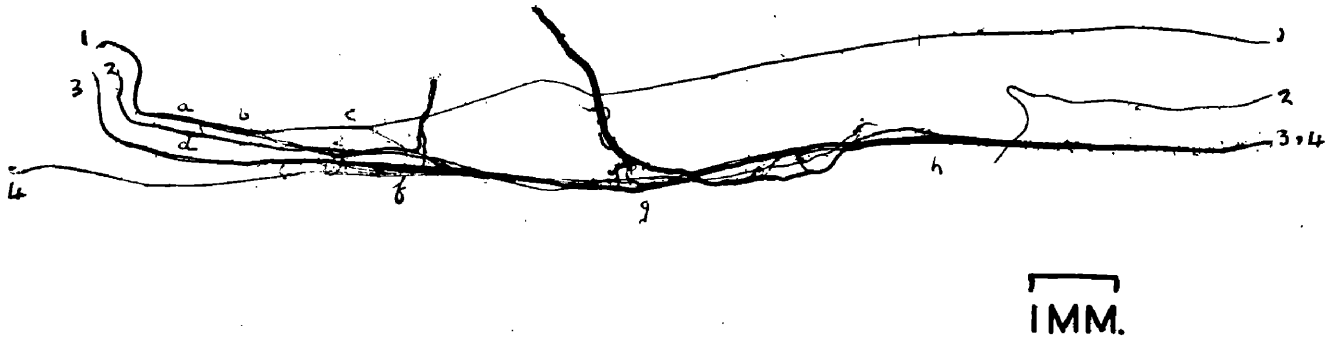

Fig.74. Photograph of a complete spindle system in m. ext. long. dig. IV. The muscle was stained with gold chloride and the extrafusal muscle fibres removed by teasing in glycerine. The intrafusal muscle fibres lie in bundles (1-4). The nerve endings are marked. a, d, f, g and h are sensory regions, while b, c and e are motor endings.

two separate groups of fibres will unite to pass through a single sensory region, e.g., in Fig.74 bundles 3 and 4 unite to pass through the sensory region, h.

Sensory nerve ending.

The form of nerve ending which was assumed to be sensory is shown in Figs. 75-78. It closely agrees with the descriptions of the spindle sensory ending given in the literature. As a large, heavily medullated, nerve fibre (18 μ diam.) approaches a bundle of intrafusal muscle fibres it divides into several branches which innervate different muscle fibres within the bundle (Figs. 75 and 76). Sometimes the nerve fibres wind round the muscle fibres (Fig.77); they then form chains of small blobs along the muscle fibres on either side of the point of nerve entry, giving a granular appearance to the muscle.

Quite commonly, a nerve fibre divides to innervate sensory regions on two different intrafusal muscle bundles (Fig.78).

Motor nerve endings.

At either, or both, sides of these sensory endings there are almost invariably other smaller endings found, e.g., at regions b and c on bundle 1 of Fig.74. It seems likely that these nerve endings may be motor in function.

In Fig.78 the ending b can be seen lying just to the

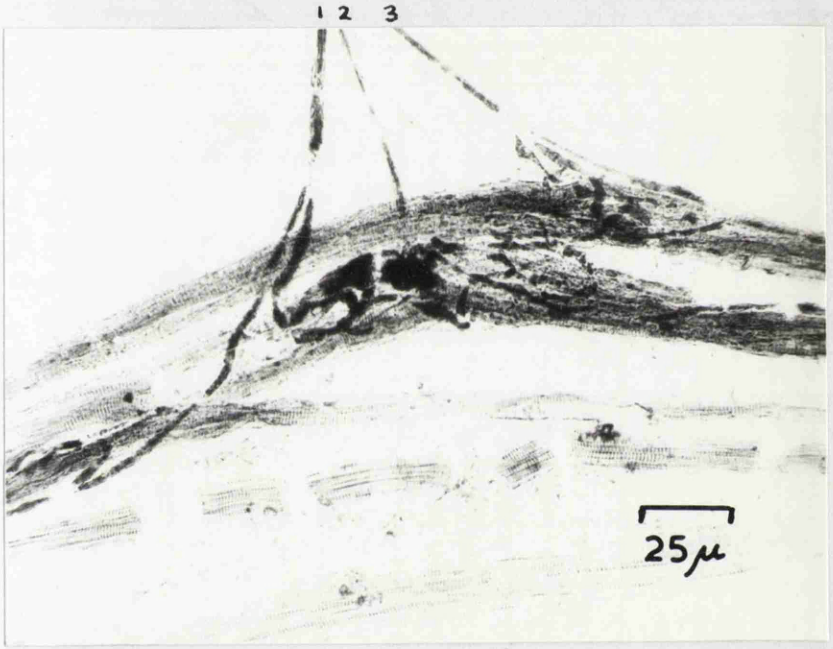

Fig.75. Photograph of sensory regions on three bundles of intrafusal muscle fibres. Two large (18 μ diam.) medullated nerve fibres (2 and 3 are branches of one axon) divide into branches which form endings on different muscle fibres. On reaching the muscle fibre each branch further subdivides many times (gold chloride).

Fig.76. A sensory region on a bundle of intrafusal muscle fibres. A large medullated nerve fibre (18μ diam.) divides up many times and forms endings on all the fibres in the bundle. The ending extends for about 0.4 mm. on either side of the point of nerve entry and takes the form of small blobs scattered over the surface of the muscle fibres (gold chloride).

Fig.77. Sensory region in which the nerve fibres coil around the intrafusal muscle fibres before breaking up to form the dotted endings (gold chloride).

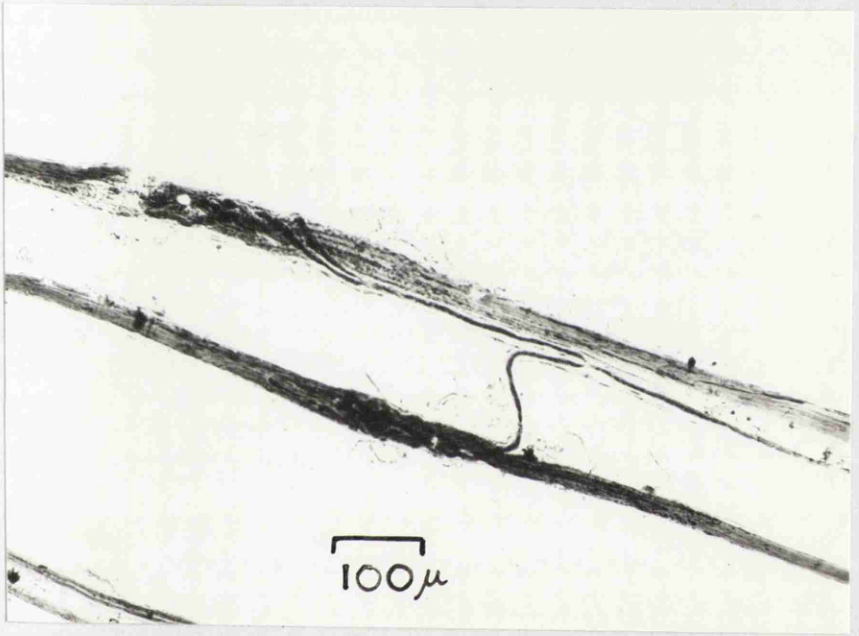

Fig.78. Sensory regions of two different intrafusal muscle bundles innervated by branches of the one large (18μ) diameter medullated nerve fibre. These are the sensory regions a and d of Fig.74. The motor ending b is seen to the right of the region a (gold chloride).

right of the sensory region a. It is innervated by a finely medullated fibre of about 7 μ diameter. Part of this ending b is shown at a higher magnification in Fig.79. Bundle 1 consists of 3 muscle fibres - one large fibre (14 μ diam.) and two small fibres (4 μ diam.). At the region b the two smaller muscle fibres lie over the larger muscle fibre. The nerve ending appears to be on the outside edges of the muscle bundle and it may be that it only innervates the smaller fibres. It is, however, impossible to be sure that the large fibre is not also innervated by the same nerve fibre.

The region c of bundle 1 is shown under higher magnification in Fig.80. Here the ending is unbranched and plate-like in form and lies only on the large fibre. It is supplied by a medullated nerve fibre of about 10 μ diameter.

In Fig.81, a bundle composed of one large (16 μ diam.) and one small muscle fibre (6 u diam.) is shown. Here the ending is on the small fibre only. It resembles the grape motor endings found on the small extrafusal muscle fibres by Gray (1957).

Intrafusal muscle fibres.

As mentioned above, the intrafusal muscle fibres run parallel to the extrafusal ones and from end to end of the muscle. They show marked cross striations which are lost in the sensory regions.

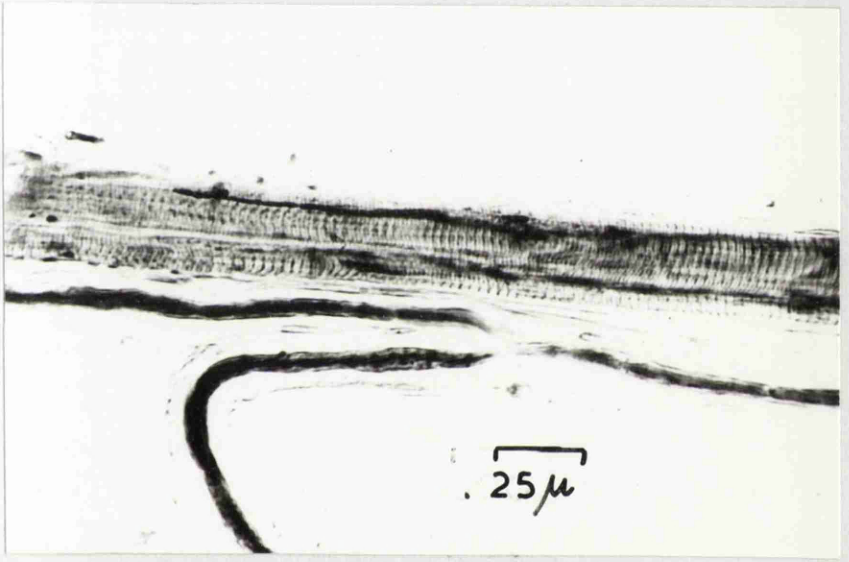

Fig.79. Part of the motor ending b of Figs. 74 and 78 under higher magnification. This bundle of intrafusal muscle fibres contains one large and two smaller fibres which are here lying over the larger one. The nerve ending appears to be at the outside edges of the bundle and may only innervate the smaller fibres. The nerve fibre is that innervating sensory regions a and c. The terminal blobs of ending a can be seen at the extreme left of the photograph (gold chloride).

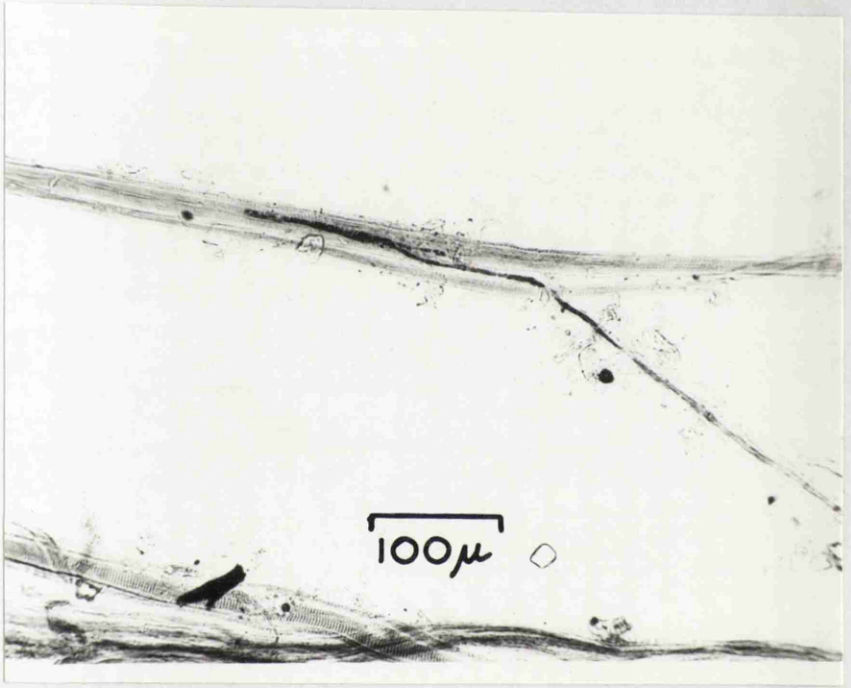

Fig.80. The motor ending c of Fig.74 under higher magnification. This ending lies only on the larger intrafusal muscle fibre (14 μ) (gold chloride).

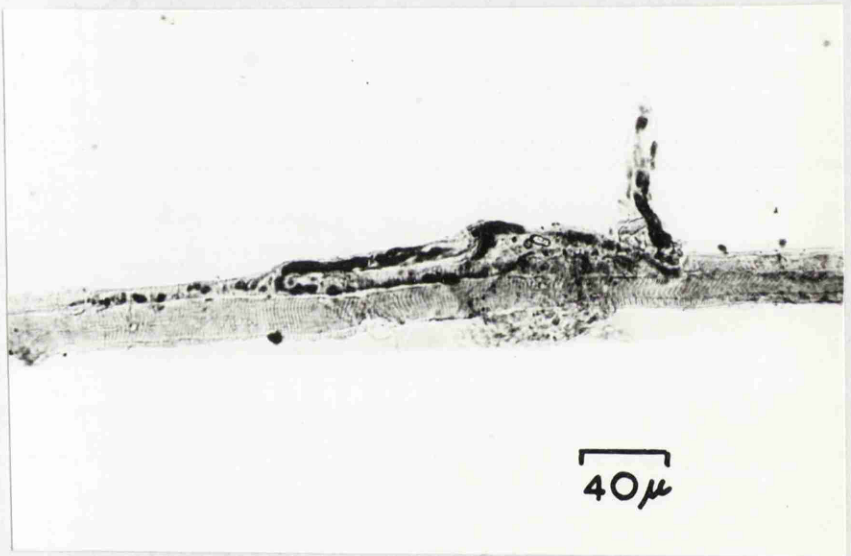

Fig.81. A bundle of intrafusal muscle fibres containing one large (16 μ) diam. muscle fibre and one small (6 μ) diam muscle fibre. A nerve ending similar to the grape motor endings on the tonic extrafusal fibres is seen on the smaller muscle fibre. The edge of a sensory region can be seen at the right of the photograph (gold chloride).

The diameters of the intrafusal fibres range from 4 μ to 16 μ. The fibres composing one bundle within the spindle system are often of very different sizes. Fig.82 shows a bundle composed of one large (14 μ) and two small (4 μ) diameter fibres.

These differences in diameter appear to be maintained throughout the length of the fibres and are not due to tapering at the ends of the muscle.

It appears possible that the intrafusal muscle fibres might be divisible into a group of large and a group of small diameter fibres. To obtain more information about fibre diameter two muscles were stained with haemalum and eosin and serially sectioned throughout their length.

The muscles were fixed in situ and then dissected out and stained. After staining they were cut in serial sections of 7 μ thickness. About 800 sectionswere made for each muscle. These were examined and at every 16th section the intrafusal muscle fibres within the bundles were drawn and measured, giving about 50 measurements of the diameter of each fibre along the length of the spindle bundles.

These measurements, in addition to giving information about the number and diameter of the intrafusal muscle fibres, also showed the position of the sensory regions on each spindle as indicated by the presence of the lymph spaces. Fig.83 shows diagrams of the lay-out of the sensory regions

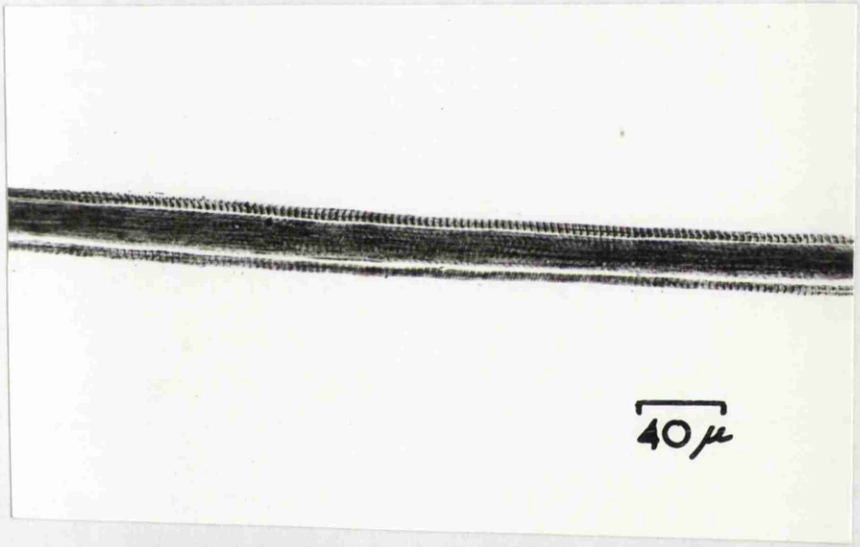

Fig.82. A bundle of intrafusal muscle fibres (bundle 1 of Fig.74) showing the difference in fibre size and the marked cross striations (gold chloride).

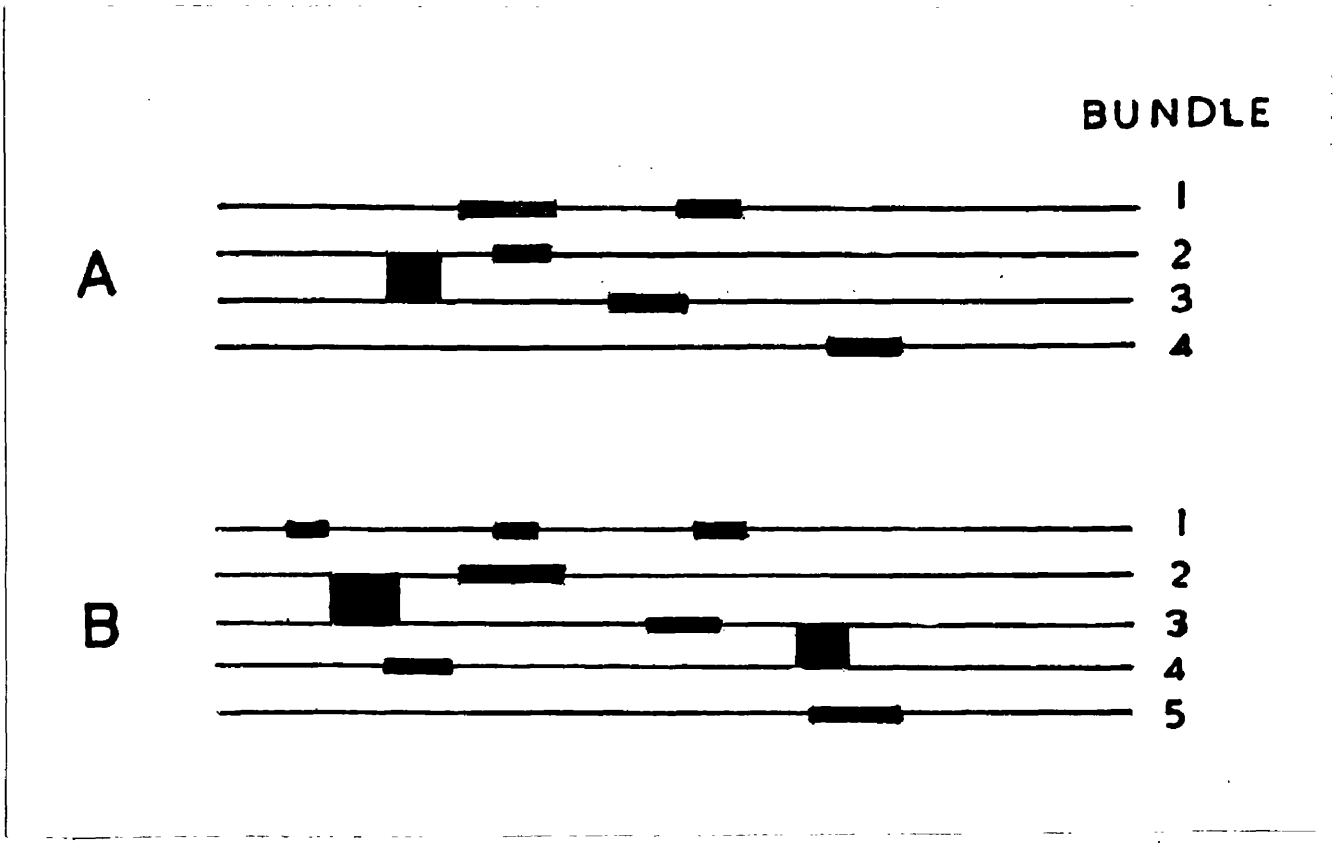

Fig.83. The position of the sensory regions on the bundles of intrafusal fibres of the spindle systems of muscles A and B.

Each blocked area represents a sensory region as indicated by the presence of a lymph space round the intrafusal fibres in the cross-section of the muscle.

of the spindle systems in both muscles. The picture is similar to that obtained from muscles stained with gold chloride.

Fig.84 shows the separation of the intrafusal muscle fibres into bundles lying among the extrafusal fibres.

The measurements of fibre diameter for one muscle are shown in Table 6. This muscle contained four bundles of intrafusal fibres with a total of 16 muscle fibres.

The diameter of any fibre remained fairly constant throughout the length of the spindle. In the sensory regions the muscle fibres were packed with nuclei and surrounded with nerve fibres so that it was more difficult to obtain an accurate measure of their size. The smaller fibres appeared to become swollen in this region, while the larger fibres were somewhat constricted. There was some tapering of the muscle fibres at the ends of the spindle, although the relative sizes of the fibres within any bundle remained the same.

The maintenance of the differences in diameter of the muscle fibres within a spindle bundle is illustrated in Figs.85-86. In these figures are shown cross sections of muscle A at equal intervals along its length. Bundle IV is composed of one large and two small muscle fibres which maintain their relative sizes throughout the length of the spindle.

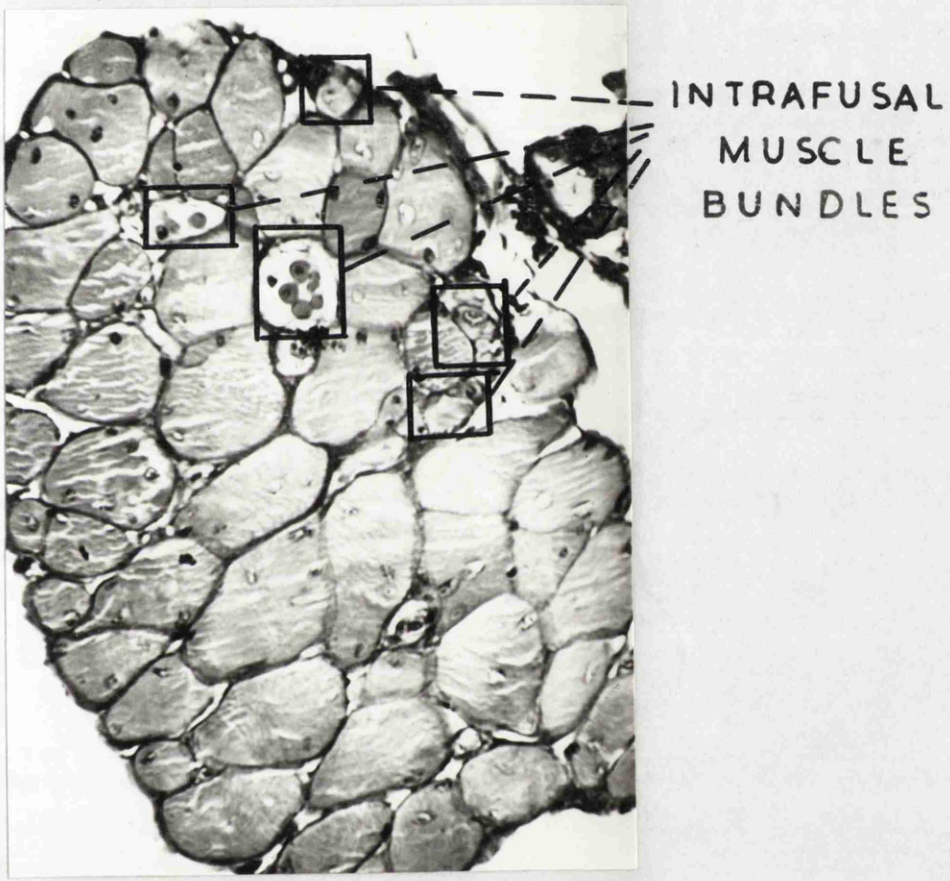

Fig.84. A cross-section of muscle B. The intrafusal muscle bundles are marked. It can be seen that, although they lie fairly close together, the bundles are quite separate (H and E).

Table 6.

Table showing the diameters of cross-sections of the intrafusal muscle fibres of muscle A. The fibres were traced throughout the length of the muscle. Each slide carried about 16 cross-sections of the muscle. The diameter of the intrafusal muscle fibres were measured in one cross-section on each slide.

Diameter of intrafusal muscle fibres (μ)

	Bundle I					Bundle II				Bundle III				Bundle IV		
Fibre	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3
Slide No.																
5	6	11	3	3	9	19	2	-	6	18	3	2	7	20	8	7
6	7	11	5	4	9	21	4	4	12	22	3	2	9	17	8	8
7	7	11	4	4	11	26	4	5	13	24	3	3	5	18	8	6
8	8	11	4	4	9	23	6	6	10	20	5	3	9	17	7	6
9	11	13	4	4	10	20	3	3	8	15	3	3	6	20	7	6
10	11	12	6	4	11	17	5	5	7	15	4	5	9	18	5	6
11	10	13	5	5	11	11	5	6	8	15	4	9	10	15	7	7
12	9	10	6	4	9	13	5	5	7	16	5	7	9	18	6	5
13	9	9	5	5	8	18	5	4	9	18	4	6	8	19	6	6
14	7	5	5	6	6	11	4	4	7	18	3	4	10	17	6	6
15	7	7	6	6	7	14	5	5	7	18	5	5	9	18	5	5
16	9	6	5	5	6	14	5	5	7	17	4	5	13	18	4	5
17	9	4	6	4	7	11	6	7	10	20	5	5	7	16	4	5
18	11	5	8	6	10	20	7	7	7	20	6	6	9	22	6	4
19	11	6	7	7	8	18	4	6	10	18	5	4	8	20	5	4
20	12	5	6	6	8	20	8	6	10	18	4	4	9	22	5	6
21	11	6	5	5	9	25	8	6	10	23	8	6	12	25	6	6
22	13	7	5	6	9	16	5	6	7	14	5	4	7	25	6	4
23	17	6	5	6	10	20	5	6	10	14	6	5	11	25	3	4
24	11	5	6	6	7	20	5	6	7	14	7	4	7	26	3	5
25	11	4	4	5	8	20	5	6	7	21	6	6	11	28	4	5
26	10	5	5	6	10	20	6	6	10	27	6	6	12	29	5	5
27	14	9	7	6	12	18	6	6	11	30	6	10	11	30	6	8
28	12	14	5	6	12	18	6	6	9	30	8	6	10	30	8	9
29	11	10	5	5	13	16	7	4	10	35	6	6	11	25	10	7
30	11	7	5	5	15	16	6	6	9	25	6	6	9	15	7	11
31	11	10	5	4	15	16	6	5	7	24	7	7	8	15	7	9
32	10	10	5	6	15	20	5	5	8	22	5	5	9	12	5	5
33	12	10	3	3	7	17	5	6	6	19	7	6	6	9	5	6
34	11	10	4	4	7	12	5	4	6	22	4	4	9	13	5	6
35	16	10	4	4	6	15	6	6	4	15	4	5	9	11	4	4
36	16	8	4	4	6	14	6	6	6	16	5	5	8	13	7	9
37	14	6	4	5	6	11	6	5	6	13	4	4	6	11	5	5
38	17	5	4	4	6	10	6	4	5	11	4	4	7	17	7	10
39	13	4	4	3	4	9	7	5	6	10	5	5	6	26	5	5
40	12	4	4	4	3	12	5	4	5	13	4	4	5	28	5	6
41	12	4	2	4	4	10	6	5	6	9	4	2	5	23	5	7
42	9	4	4	4	4	10	6	5	6	9	4	2	5	23	3	4
43	9	4	4	4	3	10	6	5	6	10	5	4	4	20	3	4
44	9	2	3	4	3	9	6	4	6	9	5	-	6	20	4	5
45	8	4	4	3	2	8	5	4	7	9	5	-	7	20	3	4
46	6	3	3	3	3	9	6	5	7	9	5	-	5	22	3	4
47	6	3	3	3	4	8	6	4	6	9	4	-	4	20	4	4
48	5	4	4	4	-	9	5	4	5	7	-	-	4	17	3	4
49						8	5	5	6	10	-	-	4	14	2	5
50						8	4	4	5					10	1	2
Average diam of fibres	10	7	4.5	5	8	15	5	5	7.5	16.5	5	5	8	19	.5	5.5

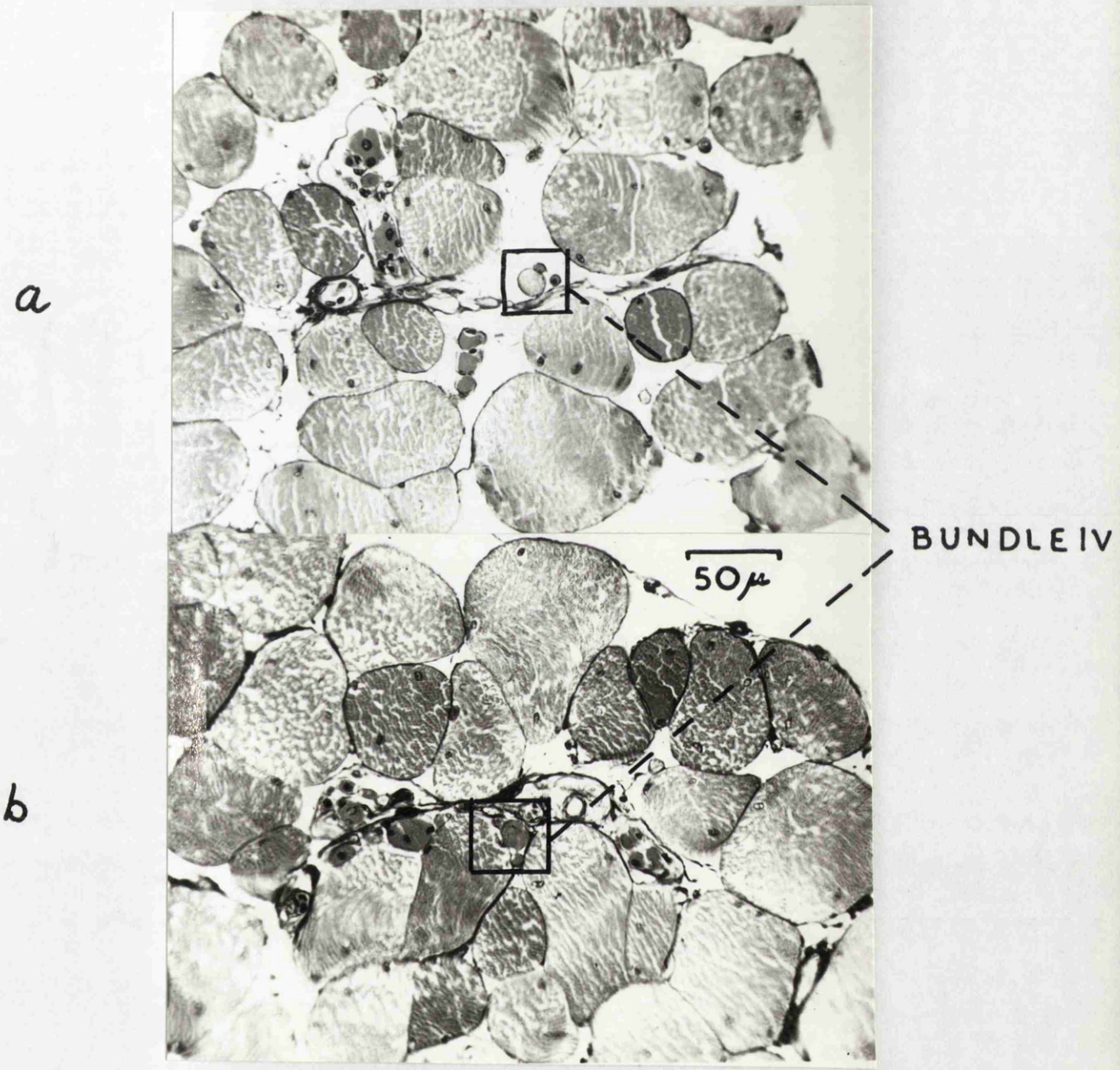

Fig.85. Photographs of cross-sections of muscle A in which intrafusal bundle IV is marked. This bundle is composed of one large and two small diameter muscle fibres and cross sections were taken at equal intervals along the length of the muscle to illustrate the maintenance of the fibre size differences within the bundle (H & E).

a) Cross section taken one fifth from end of muscle.
b) Cross section taken two-fifths from end of muscle.

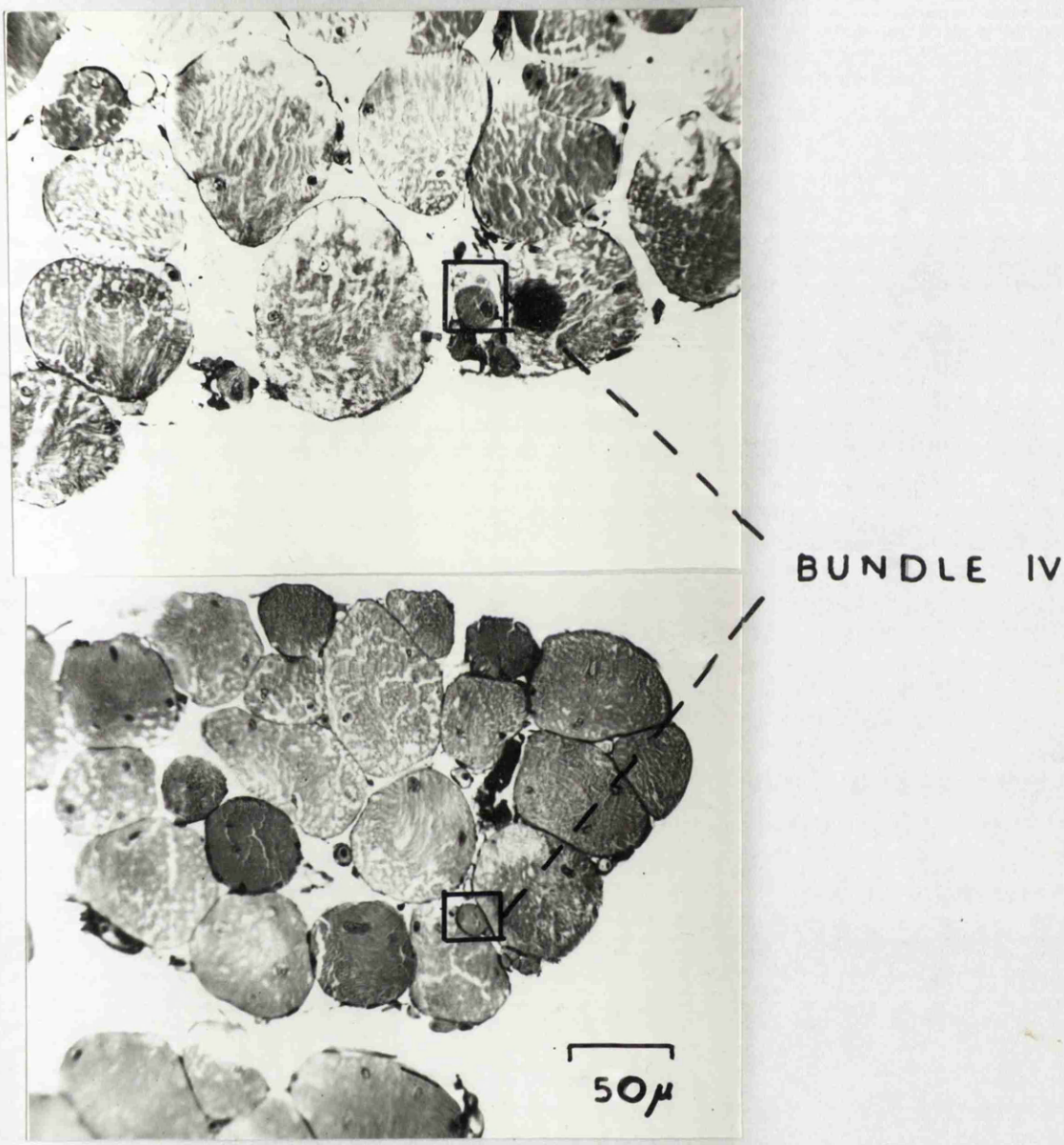

Fig.86. Similar cross sections of muscle A as are shown in Fig.85.

a) Cross section taken three fifths from end of muscle.
b) Cross section taken four fifths from end of muscle.

Since there is slight tapering of the intrafusal fibres at both ends of the spindle, average values of the diameter of the fibres, taken from all the measurements, do not give a completely true picture of fibre size, e.g., for muscle A.

Bundle	I					II				III				IV		
Fibre	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3
Average value of fibre size (μ)																
a) Including all measurements	10	7	4.5	5	8	15	5	5	7.5	16.5	5.5	8		19	5	5.5
b) Omitting measurements at end of muscle	11	10	5	5	8.5	16	5	5.5	7.5	17	5.5	9		20	5.5	6

Muscle B contained 5 bundles of intrafusal fibres with a total of 24 muscle fibres.

The average values of fibre size for this muscle (omitting tapering ends) were:

Bundle	I		II				III						IV						V				
Fibre	1	2	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5
(μ)	10	4.5	6	4	12	4	4	5.5	6.5	4	8	3	3	8	4	4.5	9	3	5	9	4	4	3	1

From the two muscles, the average diameter of a total of 40 intrafusal muscle fibres was measured. A histogram of fibre size is shown in Fig.87. This shows that 22 of the fibres are less than 6 μ in diameter, and 13 are greater than

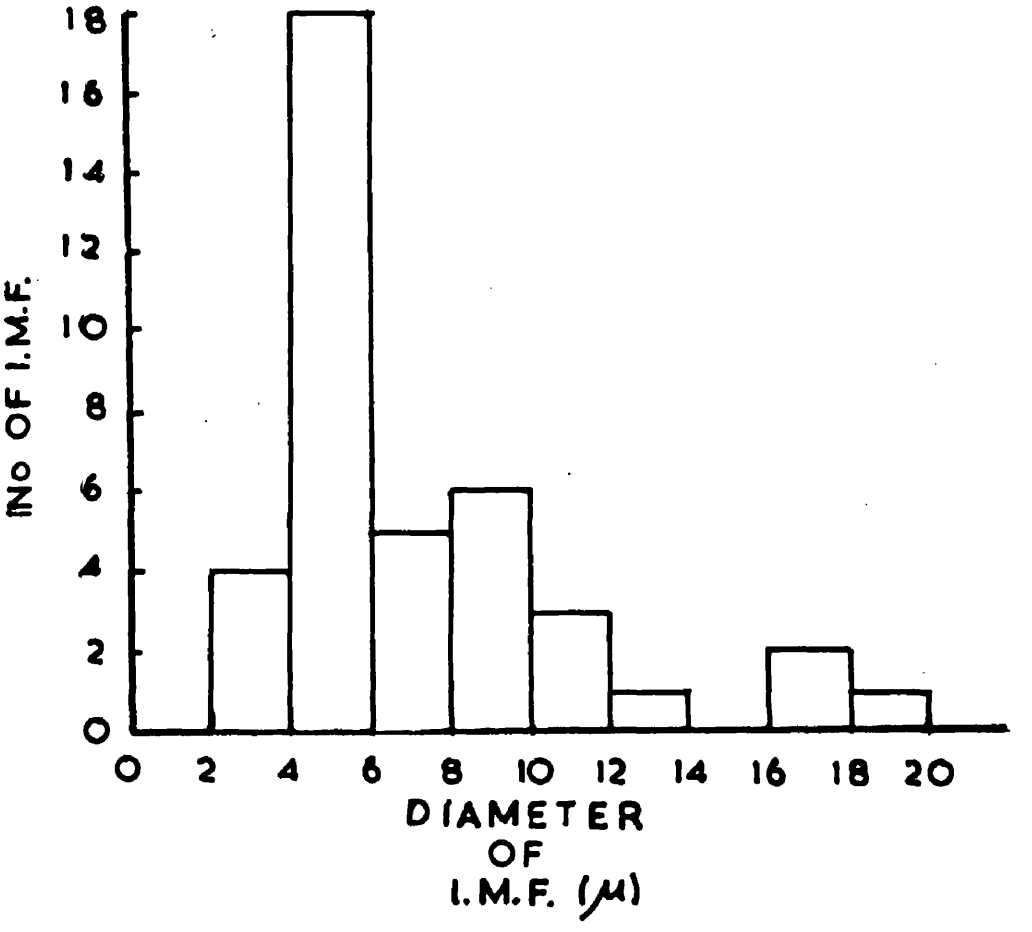

Fig.87. Histogram of fibre diameter of the intrafusal muscle fibres (i.m.f.) in muscles A and B.

8 μ in diameter. From the histogram the distribution of fibre size is skew and shows some signs of bimodality.

While the number of measurements made is too small to show conclusively that there are two distinct sizes of intrafusal muscle fibre, there are fairly strong indications that this may be so.

Discussion and Conclusions.

From study of the muscles stained with gold chloride it appears that in m. ext. long. dig. IV the spindle system is composed of several bundles of intrafusal muscle fibres. Different sensory regions, on one, or more, bundles may be innervated by branches of a single nerve fibre.

In addition to these sensory nerve endings two other types of nerve ending have been seen on the intrafusal muscle fibres. These are thought to be motor nerve endings. In this case these two types of ending were found on different sizes of intrafusal muscle fibres. The 'plate' ending appears to be found on the larger intrafusal muscle fibres (Fig.81), while the 'grape' ending is seen on the smaller muscle fibres (Fig.82).

Examination of the serial sections of two muscles gives some support to the idea that there may be two distinguishable groups of intrafusal muscle fibres present in the spindle.

Physiological studies on the frog muscle spindle give results which are in agreement with this picture of the spindle structure.

In Matthews (1931b) second paper on the properties of the frog muscle spindle in m. ext. br. prof. dig. III he observed that when the motor nerve fibres were stimulated with a stimulus of twice maximal strength, the afferent response from the spindle continued during the twitch of the

extrafusal fibres, i.e., during the so-called silent period. This result was only obtained on stimulation of certain nerve fibres. It could be accounted for if some of the intrafusal muscle fibres were innervated by high threshold nerve fibres and the resulting intrafusal contraction stimulated the nerve endings.

The diameter of the fibres in the ventral root of the frog show a bimodal distribution with a corresponding difference in function (Tasaki & Mizutani, 1944; Tasaki & Tsukagoshi, 1944; Kuffler & Gerard, 1947; Kuffler, Laporte & Ransmeier, 1947). Stimulation of the larger motor axons (12 μ) leads to a muscle twitch with a propagated muscle action potential, while stimulation of the smaller nerve fibres (5 μ) results in a non-propagated local potential change with a much slower localised muscular contraction. Kuffler & Vaughan Williams (1953), by selective stimulation and intracellular recording, have found that the two sizes of nerve fibre innervate two distinct muscle fibre groups.

Katz (1949), using m. ext. long. dig. IV, recorded the effect on the afferent discharge from the spindle of stimulation of the two types of motor axon. On stimulation of the large motor axons with a threshold stimulus, he found that, during isometric contraction of the muscle, the discharge from the spindle increased. The intrafusal and extrafusal effects could be separated by the differential action of curarine.

On curarising the muscle until, on stimulating the large motor axons, the muscle twitch was abolished and only the end plate potential remained, the sensory response from the spindle was undiminished. When single large motor axons were stimulated, most, but not all, caused an increase in the spindle response. On small nerve stimulation, about 50% of the fibres gave an increased spindle discharge. Thus, from Katz' work, it would appear that, in the frog, the intrafusal muscle fibres are innervated by branches of the motor axons which supply the extrafusal fibres.

Eyzaguirre & Vial (1956) by intracellular recording in the frog spindle in m. ext. long. dig. IV, showed the presence of intrafusal muscle fibres with a propagated muscle action potential - 'twitch' fibres.

As mentioned above, in the frog, corresponding to the two types of motor axon, there are two groups of extrafusal muscle fibres. Those innervated by the larger motor axons have a propagated muscle action potential - the twitch system - while those innervated by the smaller motor axons show non-propagated potential changes and local contractions in the regions of the motor nerve endings - the tonic system. Günther (1949) believes that there are two types of motor nerve ending, those on the twitch fibres are ordinary motor end plates, while those on the tonic fibres are grape endings.

Katz has shown that, in the frog, the intrafusal muscle fibres are innervated by branches of the nerve axons supplying the extrafusal muscle fibres. These extrafusal muscle fibres fall into two groups. It may be that there are also two types of intrafusal muscle fibre.

There is some functional evidence for this assumption. Koketsu & Nishi (1957) have made intracellular recordings from intrafusal muscle fibres in m. ext. long. dig. IV. They have found two types of response to nerve stimulation.

a) A propagated action potential. A large potential change which overshoots the resting potential and is all-or-none.

b) A potential which is small compared with the resting potential. They call it the intrafusal junctional potential, i.j.p. This i.j.p. showed fluctuations in its rising phase, and varied with electrode position, suggesting that it was a non propagated local electrical response.

Their basic assumption appears to be that there is only one type of intrafusal muscle fibre, and from its electrical characteristics they attempt to determine whether it resembles twitch or tonic extrafusal muscle fibres. From reading their paper, it would appear that a more satisfactory interpretation of their results would be on the basis of there being two types of intrafusal muscle fibre.

Until recently there was very little histological evidence for the motor innervation of the frog neuromuscular spindle. However, shortly after this work was begun, Gray (1957) published his study of the innervation of the spindle system and of the extrafusal muscle fibres in m. ext. long. dig. IV, using methylene blue and osmic acid.

In his study of the extrafusal innervation, he found that the small extrafusal efferent nerve fibres ended as 'grape' end-plates, several of which could occur on one muscle fibre - the tonic system. The large nerve fibres ended, on muscle fibres not innervated by the smaller axons, in 'Endbüschel' end plates - the twitch system. He measured the diameter of the extrafusal muscle fibres and found that the tonic muscle fibres tended to be of smaller diameter than the twitch ones.

Twitch fibres	30-120 μ.	Peak value 60-70 μ.
Tonic fibres	10-80 μ.	Peak 40-50 μ.

Gray found that each muscle contained 2 or 3 bundles of intrafusal fibres. Each bundle had a number of encapsulated sensory regions in series along it. The intrafusal bundles were not completely separate; the fibres from one bundle often passed through an encapsulated sensory region along with fibres mainly from another bundle.

Between the sensory regions were motor endings, both grape and twitch, which were derived from nerves which also

supplied the extrafusal fibres. The two types of motor ending were never both seen on the same muscle fibre.

The work reported in this thesis, while on a much smaller scale, confirms most of Gray's findings (with different staining methods).

The lay-out of the spindle system was found to be the same except that a less regular innervation pattern was found. Gray represents the sensory and motor regions as being regularly distributed along the length of intrafusal fibres. As can be seen in Fig.74, in the gold chloride stained specimens, an intrafusal muscle bundle often appeared to be innervated only at one end.

The sensory nerve ending is shown to have a form similar to that shown by Gray: a large medullated nerve fibre divides up into branches, often coiling round the muscle fibres, and forms long dotted endings on the intrafusal muscle fibres.

Two types of motor nerve ending are also shown in this thesis. While it may not be a general finding, from the work reported here it appears that the grape type of ending is found on a small intrafusal muscle fibre, whereas the twitch-type ending is seen on a much larger fibre.

Gray has shown that the extrafusal muscle fibres are of two sizes, the twitch fibres being of a larger diameter than the tonic ones. From the measurements of the diameters

of the intrafusal muscle fibres shown in this thesis it seems possible that they may also be divisible into a group of large and one of smaller fibres.

Since the intrafusal muscle fibres receive their motor innervation from branches of the axons supplying the extrafusal fibres, there are indications that there may be two types of intrafusal fibre functionally organised in a similar way to the organisation of the extrafusal fibres.

There is no indication, so far, that there is more than one type of sensory ending present on the intrafusal muscle fibres.

Summary.

1. The muscle was stretched with extension steps of constant velocity and the effect on a 'single-unit' response recorded.

2. This response was analysed to determine a transfer function relating the spindle discharge to the stimulating movement and to see whether this transfer function was similar to that derived for the proprioceptor of the knee-joint of the cat. This transfer function postulated that the response was due both to position and to some memory of the velocity of the movement by which this position was reached. It was thus composed of two parts, a displacement component $\Psi(s)$ and a velocity component $\Phi(v)$ The velocity component was further subdivided into 3 parts - one negative. The displacement component was constant in any position and varied linearly with position. The velocity component was exponential in form with a time constant of decay τ_i and an amplitude parameter ϕ .

3. In order to carry out the analysis accurately it was shown that the determination of the displacement component $\Psi(s)$ should be fairly precise.

4. The parameters ϕ_i $(i = 1,2,3)$ were plotted against v (the velocity of stretch) and shown to increase linearly

with v, $\phi_i = k_i v$

5. The parameters τ_i $(i = 1, 2, 3)$ were found to be independent of velocity and approximately constant for any unit.

6. The effect on the response of sinusoidal variations in position at three different speeds was recorded. By means of the transfer function, and using the parameters derived from the analysis of the response to the constant velocity steps, the expected response was calculated and compared with the experimental one. The experimental response fell away more quickly than the calculated one during the latter half of the cycle.

7. Steps of increasing tension were applied to the muscle and the responses analysed in a similar manner to the analysis of the constant velocity extension steps. As good a fit of the experimental results was obtained here as was found for the extension steps.

8. The length and tension of the muscle were recorded during sinusoidal movements at different speeds. It was found that, for any particular length of the muscle, the difference between tension during extension and during relaxation increased with speed.

9. It is suggested that the deformation at the sensory terminals, which is responsible for the spindle response,

may be directly related to the tension in the-whole muscle rather than to the extension of the muscle. If it is assumed that the recorded response to sinusoidal movements is directly related to the tension in the muscle, and at various points in the cycle this response is correlated with that calculated from the transfer function on the basis of length changes, then a relationship between the length and tension of the muscle is obtained which is similar to that determined directly.

10. 30 muscles were stained with Gairns' (1930) modification of the gold chloride technique and teased in glycerine. 2 muscles were stained with haemalum and eosin and serially sectioned.

11. The gold chloride technique showed that, as found by Gray (1957) using methylene blue, the spindle intrafusal muscle fibres were arranged in bundles and carried three types of nerve ending. A large myelinated nerve fibre divided up as it reached the intrafusal muscle fibres, sometimes coiling round them, and ended as a series of small blobs along the fibre. This was the sensory ending. Two types of motor ending were seen. An ending somewhat similar to the grape ending on the extrafusal fibres was here seen on the smaller intrafusal muscle fibres. On the large intrafusal muscle fibres an ending like the Endbuschel type

of motor ending was seen.

12. From the H. & E. stained serial sections it appeared that the diameters of the intrafusal muscle fibres were not uniformly distributed over the range of fibre size. There seemed to be a group of small fibres (less than 6 μ diam.) and possibly one of larger fibres (greater than 8 μ diam.).

13. It is suggested that corresponding to the two systems of extrafusal muscle fibres in the frog - the twitch system of larger muscle fibres and ordinary motor end plates, and the tonic system with smaller muscle fibres and grape end plates - there may also be two systems of intrafusal muscle fibres functionally organised in a similar way to the extrafusal ones.

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References.

Blix, M. (1892). Die Länge und die Spannung des Muskels. Skand.Arch.Physiol., 4, 399-409.

Boyd, I.A. & Roberts, T.D.M. (1952). Proprioceptive discharges from stretch receptors in the knee-joint of the cat. J.Physiol., 122, 38-58.

Bronk, D.W. (1929). Fatigue of the sense organs in muscle. J.Physiol., 67, 270-281.

Buchthal, F. (1942). The mechanical properties of the single striated muscle fibre at rest and during contraction, and their structural interpretation. D.Kgl.Danske Vidensk.Selskab. Biol.Medd., 17, 2.

Buller, A.J., Nicholls, J.G. & Ström, G. (1952). Spontaneous fluctuations of excitability in the muscle spindle of the frog. J.Physiol., 122, 409-418.

Cajal, R. (1888). Terminaciones nerviosas en los husos musculares de la rana. Rivista trim. Histol. Norm. y Patol., vol.1, fasc.1.

Davey, Mary R. & Roberts, T.D.M. (1958). A transfer function relating deformation to impulse frequency for a muscle spindle in the frog's toe. J.Physiol., 143, 16P.

Eyzaguirre, C. & Vial, J.D. (1956). Electrical activity of the intrafusal muscle fibres. Nature, 178, 317-318.

Gairns, F.W. (1930). A modified gold chloride method for the demonstration of nerve-endings. Quart.J. Micr.sci., 74, 151-153.

Gray, E.G. (1957). The spindle and extrafusal innervation of a frog muscle. Proc.Roy.Soc.B., 146, 416-430.

Hines, H. (1930). The innervation of the muscle spindle. Proc.Ass.Res.Nerv.Ment.Dis., 9, 124-152.

Huber, C. & De Witt, L. (1898). A contribution on the motor nerve-endings and on the nerve-endings in the muscle-spindles. J.Comp.Neurol., 17, 169-230.

Katz, B. (1949). The efferent regulation of the muscle spindle in the frog. J.Exp.Biol., 26, 201-218.

Katz, B. (1950a). Action potentials from a sensory nerve ending. J.Physiol., 111, 248-260.

Katz, B. (1950b). Depolarisation of sensory terminals and the initiation of impulses in the muscle spindle. J.Physiol., 111, 261-282.

Koketsu, K. & Nishi, S. (1957). Action potentials of single intrafusal muscle fibres of frogs. J.Physiol., 137, 193-209.

Kolliker, A. von (1862). On the termination of nerves in muscles. Proc.Roy.Soc., 12, 65-84.

Kuffler, S.W. & Gerard, R.W. (1947). The small-nerve motor system to skeletal muscle. J.Neurophysiol., 10, 383-394.

Kuffler, S.W., Laporte, Y. & Ransmeier, R.E. (1947). The function of the frog's small-nerve motor system. J.Neurophysiol., 10, 395-408.

Kuffler, S.W. & Vaughan Williams, E.M. (1953). Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle and the membrane characteristics of the fibres they innervate. J.Physiol., 121, 289-317.

Matthews, B.H.C. (1931a). The response of a single end organ. J.Physiol., 71, 64-110.

Matthews, B.H.C. (1931b). The response of a muscle spindle during active contraction of a muscle. J.Physiol., 72, 153-174.

Roberts, T.D.M., Boyd, I.A. & Cairnie, A.B. (1956). Derivation of a transfer function for a stretch receptor. Abstract of Communication XXth Inter.Physiol.Cong., Brussels, p.767.

Sherrington, C.S. (1894). On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibres in the ventral spinal nerve-root. J.Physiol., 17, 211-258.

Tasaki, I. & Mizutoni, K. (1944). Comparative studies of the activities of the muscle evoked by two kinds of motor nerve fibres. Part I. Myographic studies. Jap.J.Med.Sci., 10, 237-244.

Tasaki, I. & Tsukagoshi, M. (1944). Comparative studies on the activities of the muscle evoked by two kinds of motor nerve fibres. Part II. The electromyogram. Jap.J.Med.Sci., 10, 245-251.

Weismann, (1861). Ueber das Wachsen der quergestreiften Muskeln nach Beobachtungen am Frosch. Z.rat.Med., 3D Ser., 10, 263-284.

Wilkinson, H.J. (1929). The innervation of striated muscle. Med.J.Aust. vol.2, No.22, 768-793.

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A transfer function relating deformation to impulse frequency for a muscle spindle in the frog's toe. By MARY R. DAVEY and T. D. M. ROBERTS. *Institute of Physiology, University of Glasgow*

We have now applied, to a muscle spindle of the frog's toe, the same procedure for deriving a transfer function as was used for the cat's joint receptor (Roberts, Boyd & Cairnie, 1956). The general form of the transfer function for the responses of the muscle spindle to 'constant velocity' steps of extension turns out to be the same as that which had been derived for the joint receptor, namely

$$F_t = \Psi(s) + \Phi_1(v, t) - \Phi_2(v, t) + \Phi_3(v, t),$$

where $\Phi_i(v, t_1) = \int_{-\infty}^{t_1} \phi_i(v_t)\, e^{-(t_1-t)/\tau}\, \Delta t$, F_t is the impulse frequency at time t, $\Psi(s)$ is the impulse frequency at rest in a position corresponding to the deformation s, $\phi_i(v_t)\, e^{-(t_1-t)/\tau}\, \Delta t$ is a component of the total impulse frequency at time t_1 which is contributed by that particular rate-of-change of deformation v which occurred in the (previous) interval t to $(t+\Delta t)$, and τ is a time constant.

REFERENCE

Roberts, T. D. M., Boyd, I. A. & Cairnie, A. B. (1956). *Proc. XX int. Congr. Physiol.* p. 767.