

## THE FREQUENCY RESPONSE OF FROG MUSCLE SPINDLES UNDER VARIOUS CONDITIONS

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### SUMMARY

1. Nerve impulses were recorded from afferents from non-contracting spindles from the isolated extensor longus dig. IV muscle of the frog during small sinusoidal changes in muscle length at frequencies from 0.001 to 100 Hz. A computer of average transients was used to average the spike distribution during a number of cycles, and hence to determine the spindle response in impulses/sec at different phases of the cycle.

2. At any one frequency the response could be described by a sinusoid, whose amplitude was approximately proportional to the amplitude of the stretch and whose phase was approximately constant, together with a non-linearity dependent principally upon non-linearities in the static response.

3. The frequency response was estimated from the sinusoid responses. In conventional terms, it consisted of a straight line of positive slope below 2 Hz and a maximum between 7 and 16 Hz.

4. The slope of the frequency response was dependent on the mean length of the preparation, typically varying from zero to about 0.5 (3 db/octave) over the physiological range of the passive muscle. The shape of the peak appeared to depend on the mean firing frequency.

5. The responses to ramp stretches of one second duration and up to 2 mm in amplitude were also measured. Responses predicted from the sinusoid measurements were the same shape as the measured responses, but were larger by a factor of about 1.4.

6. The shapes of both the frequency responses and the responses to ramps were hardly affected by an operation that removed most of the polar parts of the spindles.

7. The results are discussed in terms of internal spindle mechanisms.

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## INTRODUCTION

A recurrent problem in muscle spindle physiology has been identification of the origin of the sensory adaptation. The problem has not been helped by the lack of exact, quantitative definition of the spindle response to stretch. Recent studies have gone some way to providing this in the case of the cat (Matthews & Stein, 1969; Poppele & Bowman, 1970; Rosenthal, McKean, Roberts & Terzuolo, 1970) but a comparable study has not yet appeared in the case of the frog where the anatomy, and possibly the function, is slightly different. The experiments described here were intended to provide some such data by using what is believed to be a particularly sensitive method, namely that of applying small, sinusoidally varying stretches and averaging the response over the stretch cycle.

Some exact measurements of frog muscle spindle responses to length changes have been made before (Davey & Roberts, 1958; Houk, Sanchez & Wells, 1962; Shepherd & Ottoson, 1965; Toyama, 1966; Ito, 1969) but the material here is new because it covers a much greater range of frequencies than were covered or implied in the results from these other experiments, together with more precision with regard to linearity and to non-linearities. The results are consistent with most of the apparently diverse results already published. The measurements presented here are also of interest because of both similarities and differences between them and those from the cat. Besides the non-linearities mentioned above and the frequency range, the results differ from the mammalian case in that the frequency response has a negative slope above about 15 Hz, i.e. the 'cut-off frequency', to use amplifier terminology, is at least 20 times lower than in the mammal. The results also differ in that the disparity between the sensitivities of cat primary endings from the small amplitude sinusoid measurements and from large amplitude ramps (Matthews & Stein, 1969), is not seen in the case of the frog.

Further experiments involved a procedure which, according to Matthews's (1931) mechanical theory, should have altered the responses to stretch. That the sensitive methods employed here failed to show such an alteration is taken as evidence against the theory, at least for this species.

One preliminary report has already appeared (Kirkwood, 1969).

## METHODS

*Preparation*

The results here were derived from the responses of seventy single muscle spindle afferents from fifty-three muscles. Reference is also made to eighteen multiple unit preparations which gave results in agreement with those from single units. The results

from a further nineteen single unit and seven multi-unit preparations were rejected because they were either not repeatable or were not checked for repeatability.

Frogs of the species *Rana temporaria*, *R. pipiens* or *R. esculenta* were used and no differences in responses were observed between species. Experiments were carried out at room temperature, normally between 20° C and 25° C. In any one experiment the temperature of the muscle bath was not allowed to vary by more than 2° C. A cooling device was available to ensure that this was so and also so that the temperature was never above 25° C. The experiments were carried out throughout the year.

Ext. long. dig. IV muscle of the frog was isolated together with a suitable length of its nerve and bathed in Ringer solution of the following composition: NaCl 112 mM, KCl 2.5 mM, CaCl<sub>2</sub> 1.8 mM, NaHCO<sub>3</sub> 1.8 mM. The surface of the Ringer solution was covered in paraffin oil and the nerve lifted on to a platinum wire electrode in the paraffin. The potential between this electrode and the pool was recorded with a conventional a.c. amplifier. Branches of the nerve which occur near the point of entry of the nerve to the muscle were cut so as to give single units. In some cases these branches were ligatured, each with a single cotton fibre and lifted on to other electrodes in the paraffin. In this way, up to three units could be separately studied at the same time.

The observed action potentials were used to trigger a 0.25 msec square pulse that was then available as a digital signal for a Computer of Average Transients (T.M.C. C.A.T. 400B).

### *Stimulation*

The muscle was mounted by means of ligatures tied on its tendons and held near the tendon in screw-clamped forceps. One pair of forceps was light and was mounted on the moving part of a moving coil vibrator (Pye-Ling V. 47), while the other was mounted on a micrometer arrangement. Thus the mean length of the muscle and variations about this length could be independently varied by means of the micrometer and vibrator respectively. The movement of the vibrator was monitored by means of a pair of semiconductor strain gauges mounted on a thin brass shim which was bent by the movement. The strain gauges were included in a bridge circuit and the voltage changes resulting from bending were found to be linear with displacement of the vibrator over the range used.

Sinusoidal voltages applied to the vibrator produced sinusoidal movements. The amplitudes of stretching (half the peak-to-peak values, as in Matthews & Stein, 1969) varied with frequency but were less than 0.05 mm at 10 Hz rising to, at the most, 0.1 mm at 100 Hz and 0.5 mm below 0.005 Hz. The amplitudes of the stretch were set and measured in each case directly from the transducer signal displayed on the oscilloscope screen.

Seventeen units in fourteen muscles were also subjected to ramp stretches of 1 sec duration so that some estimate of the extent of the range of validity of the sinusoid measurements could be made. The same vibrator was used to produce the ramps but the responses were measured, much more simply than in the case of the sines, by photographing the output of a reciprocal time-interval display unit (Huxley & Pascoe, 1963).

### *Analysis of responses to sinusoidal stretches*

The methods used for measuring the variation in firing rate over a cycle of stretch were very similar to that of Hughes & Maffei (1966) and to the probability density method of Matthews & Stein (1969). Two slightly different methods were used, covering different but overlapping frequency ranges. The first counted the spikes

occurring in each of twenty evenly spaced intervals, each of duration 0.04 times the cycle time; the other used nineteen intervals each of duration 0.05 times the cycle time. The transducer signal could also be fed into the C.A.T. via an analogue-to-digital converter so that in either method the stretch phase could be obtained from the C.A.T. data. Further measurements of the stretch phase could be made from the direct transducer signal photographed from the oscilloscope. The three methods were in mutual agreement. The response measurements from the two methods were also in agreement.

The number of cycles summated at any frequency was varied with the frequency, usually to give a constant averaging time, typically 100 or 200 sec. For stretch cycles longer than this, one or two cycles only were used and, for cases with a very low static firing frequency, longer times. An interval which varied with frequency was allowed for the response to a sinusoid to become steady before any readings were taken. The interval was about 3 min for the highest frequencies, extending to up to half an hour (two cycles at 0.001 Hz) for the low frequencies. Such intervals were found to be necessary in order to give responses that were repeatable in amplitude and phase.

### *Procedures*

Two basic experiments were carried out. In one, the mean length was held constant and the stretch frequency varied, sometimes using more than one length. In the other, the frequency was held constant (usually 0.1 Hz) and the length was varied. Other shorter experiments concerned the variation of the response with the amplitude of the stretch, although checks on this were also made during the main experiments.

The static response, that is the mean firing frequency at a given length, was always measured in the variable length experiments and measured in seventeen of the variable frequency ones. This was done by summating the response to a number of cycles but using zero amplitude for the stretch. This was a particularly convenient way because it gave twenty or nineteen separate measurements in each instance and so both the mean and standard deviation of these could be calculated. Four measurements of static response were made for each variable frequency plot, one at a length below the length at which the sinusoids were to be imposed, two at this latter length (one before the sinusoids, one after) and a fourth at a longer length. In some cases this procedure was reversed so that the static responses were recorded in decreasing order, i.e. the muscle was progressively allowed to shorten. The static sensitivity was taken as the sum of the differences between the first two measurements and between the last two, divided by the total length change. This gave a figure in impulses/sec. mm. In the case of the variable length plot, the static sensitivity at a given length was estimated as the difference between the two static responses at the two adjacent lengths to the one concerned, divided by the difference between the lengths. In these experiments the procedure was merely to record first the static response and then that to a sinusoid at each of a series of lengths.

About 20 min was always allowed for adaptation after any change of mean length. The definition of the response as 'static' after this time is arbitrarily but consistently applied in these experiments. Readings in both types of experiment were always taken in a series and return fashion (i.e. stretch frequency or length being varied monotonically first in one direction and then in the other) and the experiment was rejected if the two sets of measurements were not in agreement. An exception was made in the variable length experiments where either the static response or the amplification (see Results for definition) sometimes showed cyclic properties. The

experiment was accepted if the cycle could be repeated at least in part (see Figs. 2 and 8).

The range of lengths used in the experiments lay roughly between the slack length and the maximum length *in vivo*. It was found that the slack length was difficult to determine due to slow changes. If the length of the preparation was reduced by up to 1 mm below the length at which it first went slack, it was often found that this slackness was taken up after fifteen minutes or so. Moreover, the observed static responses were not related in a constant way to either this length or the length measured during the dissection (near the maximum *in vivo* length) although the steep parts of the characteristic always lay between these lengths. The lengths used for recording responses were therefore measured in absolute terms but the *in vivo* length during the dissection was noted. This length was of the order of 20 mm and was never exceeded by more than 1 mm. The slack length was of the order of 14 mm.

One further procedure was carried out on fourteen of the preparations (sixteen units) in order to investigate the influence of the polar parts of the spindle on the responses. After the responses of the spindle had first been measured in an intact preparation the following procedure was carried out. The screw forceps from both ends of the muscle were moved closer together and clamped tightly on either side of the presumed position of the spindle capsule (usually near the point of entry of the particular nerve branch to the muscle). The two ends of the muscle were then cut off close to the forceps, the net effect being to reduce the length of the preparation from about 18 mm to about 2 mm. Since, in this muscle, the intrafusal fibres run the whole length of the muscle (Gray, 1957; Barker & Cope, 1962), it is safe to assume that they were thus transected at two points. Immediately following this procedure, both the background discharge and the response to stretch were absent for a period ranging from 5 to 30 min. Once this period had elapsed, however (judged by the appearance of a relatively steady background discharge) responses could be recorded in the normal way and the measurements made before the operation were then repeated.

## RESULTS

### *Response to a variable stretch amplitude and fitting of sinusoids*

Fig. 1*b* and *c* shows the averaged response of a unit stretched at 0.1 Hz but at two different mean muscle lengths. The height of each dot is proportional to the number of spikes occurring in an interval equal to 0.04 times the cycle time centred at that point. The responses to two different stretch amplitudes and to zero stretch amplitude are given in each case. *a* shows the static characteristic for this unit where each point is derived from the zero amplitude plot at that length. It should be noted that at both lengths the sinusoidal stretch modulates the firing frequency above and below the resting level and also that the phase of the response leads that of the stretch.

Now, in case *b*, at a length of 20 mm, where the static characteristic is more or less linear, the response to a sinusoid is very close to sinusoidal. In this case it is easy to fit a sinusoid by eye and to estimate its amplitude and phase. In the case of the response at 18.75 mm, however, the static characteristic is not straight and the response to a sinusoid is distorted.

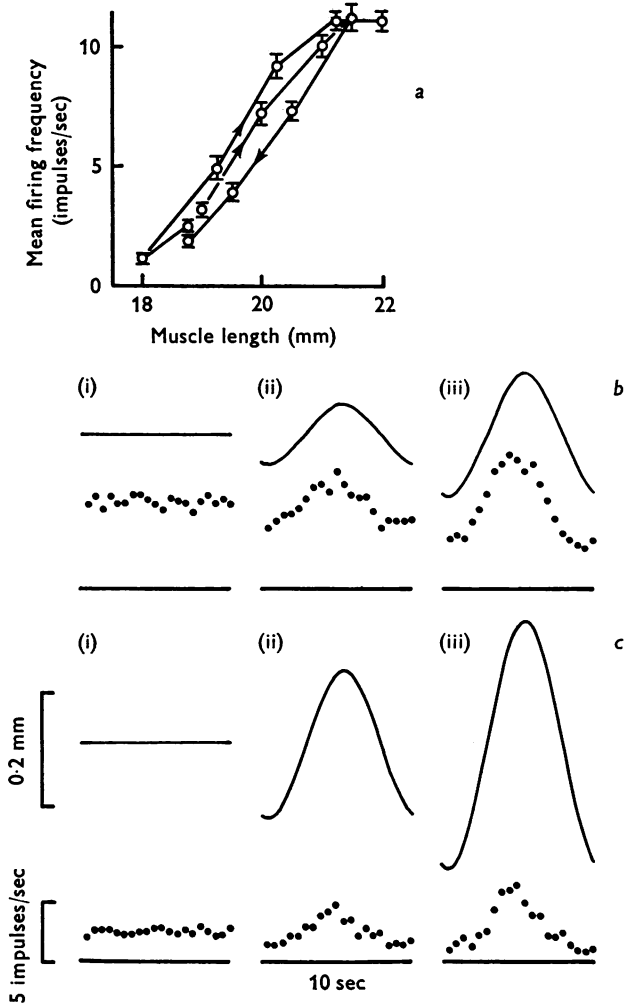


Fig. 1 *a*. Static characteristic of a spindle unit allowing 15 min for adaptation at each length. Vertical bars represent the s.d. of the twenty measurements at each point (see text) and arrows show the order of taking readings. *b*, Average response of same unit over one cycle at 0.1 Hz and at a mean muscle length of 20 mm. Upper trace: stretch; lower trace: firing frequency. *c*, As *b* but at a length of 18.75 mm. The same calibrations apply to both *b* and *c*.

Fig. 2 shows how the amplitude and phase were estimated in cases such as this. *a* is the same response as Fig. 1 *c* (iii). The procedure was first to fit a sinusoid by eye to have the same phase as the response. The height of each point,  $y_2$ , was then plotted against the corresponding ordinate  $y_1$ , of the sinusoid. The result (*b*; open circles) was a plot where the points from

ascending and descending values of  $y_2$  lay on the same curve. If this was not so, then the phase was adjusted until it was. (This was a useful check on the phase measurement.) A tangent to the curve was then drawn at the level where the width of the response peak was one half cycle. (This level

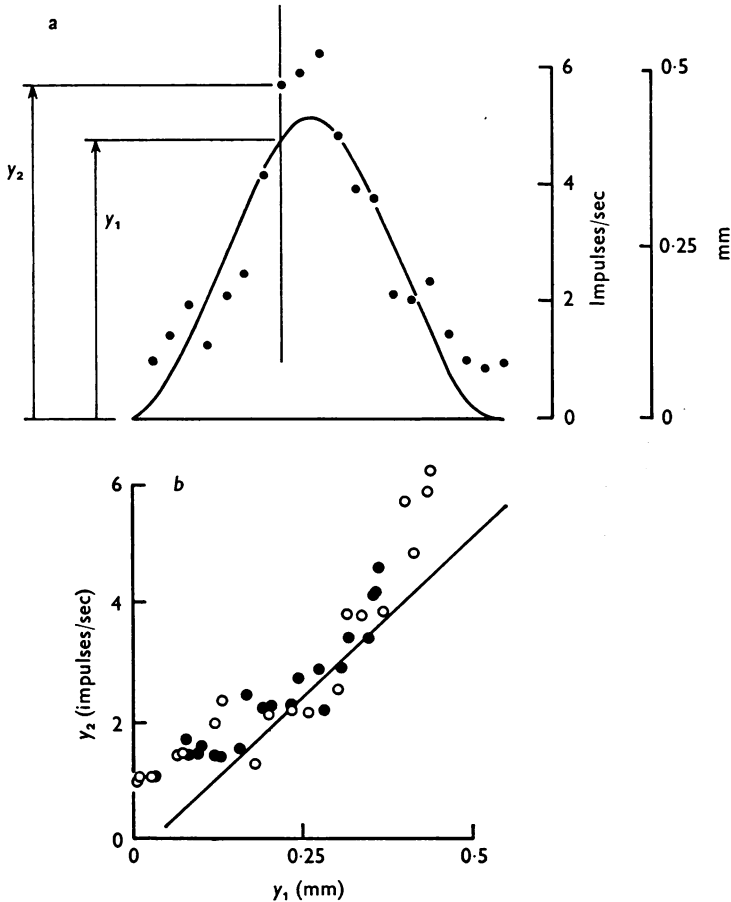


Fig. 2. Estimation of sensitivity from a distorted sinusoid. The points on *a* represent the measured response and the curve is a sinusoid with amplitude proportional to the stretch amplitude but with the phase of the response. In *b* the response ordinate,  $y_2$ , of *a* is plotted against the corresponding sinusoid ordinate,  $y_1$  (open circles). The filled circles refer to the same procedure applied to the response to a smaller stretch amplitude. The tangent was fitted by eye and its slope gives the sensitivity at this frequency.

was found to be the same as the static firing frequency.) The slope of the tangent gave the sensitivity of the unit at that stretch frequency in impulses/sec. mm.

Also included on the same plot are the filled circles, which were derived in the same way from Fig. 1c(ii). In this case the fitted sinusoid was reduced in amplitude in proportion to the stretch amplitude and had the same phase as that fitted to the larger response. It should be noted that both sets of points lie on one curve, i.e. the sensitivity was independent of the stretch amplitude. This is alternatively expressed by saying the response amplitude was proportional to the stretch amplitude. This was also true for the responses at a length of 20 mm (Fig. 1a).

This proportionality is a necessary condition for applying a linear technique such as is implied in using sinusoidal stimulation. There was insufficient time in any one experiment to test systematically for proportionality at all frequencies or lengths, but checks on the proportionality were made over the whole range of both, using several preparations. The phase was independent of amplitude within the limits of experimental error.

It should be noted that in these experiments, in contrast to those on mammalian spindles (Matthews & Stein, 1969), no silent period during a cycle was observed. This was so even with large amplitude stretches unless, that is, the amplitude was large enough for the occurrence of the spikes to become 'locked' to the stretch, a situation that was avoided. This lack of silent period can be ascribed to the lower tail of the S-shaped static response (see Figs. 1 and 8).

#### *Variation of response with frequency*

This section contains the kernel of any quantitative description which uses this method. Sixty-two plots from forty-nine units in thirty-eight muscles were analysed. In the experiments where the static sensitivity was also measured, the sensitivities were normalized by being expressed in terms of the amplification at that frequency. This is a dimensionless quantity defined as the sensitivity at a given frequency divided by the static sensitivity at the corresponding mean length (see Methods). The number thus derived is analogous both to the dimensionless gain used by Poppele & Terzuolo (1968) and to the sensitivity used by Matthews & Stein (1969). As in this latter paper, the absolute values (in impulses/sec. mm) were known but here the relative ones (the amplifications) were also known, enabling direct comparisons to be made between preparations.

Two examples of the frequency responses of spindle units are shown in Figs. 3 and 4. The parameters plotted, sensitivity versus frequency on log-log scales, are the same as one would use to define the response of an electrical amplifier or a control system. The plot of the phase versus frequency is also included, the two graphs together being the Bode Plot, familiar to automatic control engineers. Attention should first be focused



on the experimental points; the curves that are fitted will be discussed later. The predominant feature of the points for frequencies of 2 Hz or below is that the frequency response plot is approximately a straight line and the phase is constant. This was so in all cases although the slope and

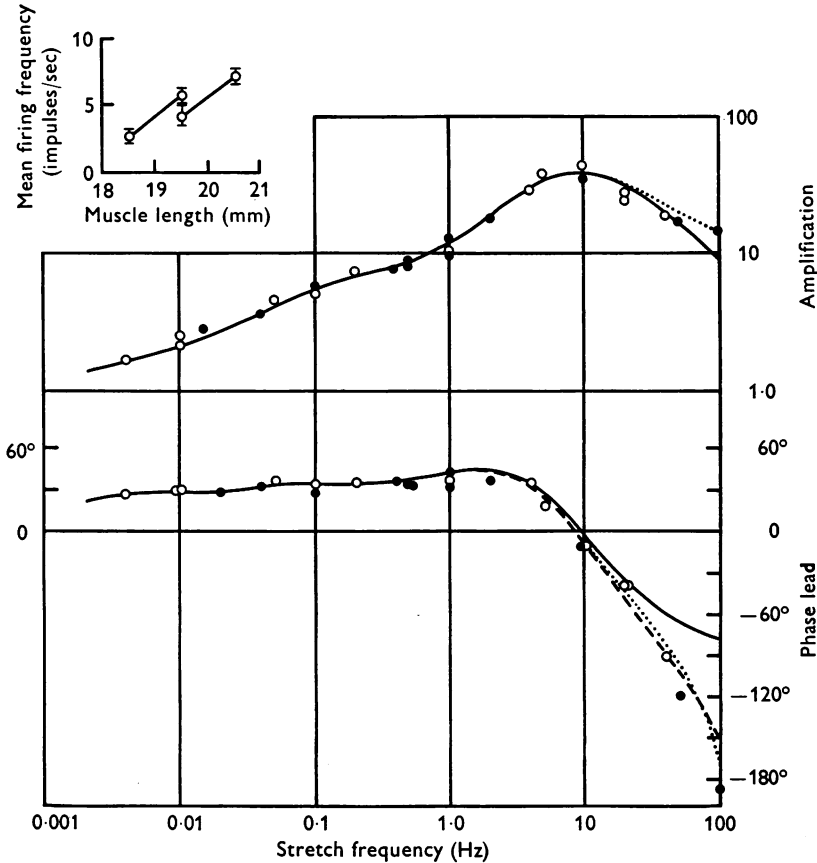


Fig. 3. Frequency response of a spindle unit. Upper curve: amplification (the units are multiples of the static sensitivity). Lower curve: phase. Filled circles: points taken with ascending values of frequency. Open circles: points taken with descending values of frequency. Inset: estimation of static sensitivity. The mean slope of the two lines is 3.0 impulses/sec. mm. Vertical bars represent the standard deviation of the twenty readings of each point (see text). The curves fitted to the frequency response are discussed in the text. In this case the damping factor of the second order response fitted to have a peak at 10 Hz is 1.03. The phase curve corresponds to a minimum phase system represented by the amplification curve. Additions to the curve above 10 Hz are (dashed line) a finite time delay of 2 msec or (dotted lines) another second order response with resonant frequency of 100 Hz and damping factor 0.4 (see text).

the phase were not the same for all preparations. (The upper curve of Fig. 4 was the most extreme case of deviation from constant phase.)

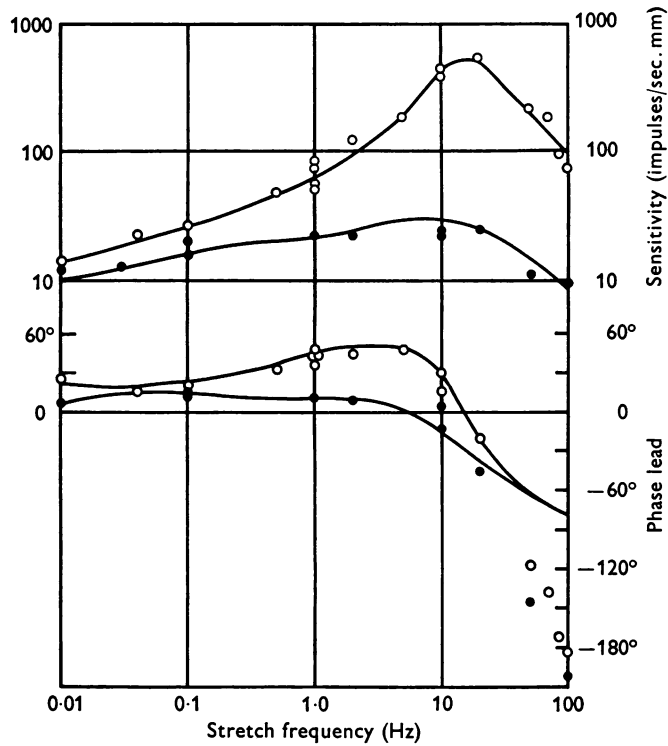


Fig. 4. Frequency response of a spindle unit at two different muscle lengths. Filled circles: muscle length 17.5 mm. Open circles: muscle length 20 mm. The fitted curves are discussed in the text. In this case the peaks were at 10 Hz and 16 Hz and the damping factors were 1.25 and 0.6 for the short and long lengths respectively.

#### *Low frequencies and comparisons with the static response*

In order to compare preparations, the frequency responses were categorized, first, by the slope,  $\alpha$ , of a straight line fitted by eye to the points below 2 Hz and by the corresponding mean phase,  $\Phi_1$ . Now, if the response is to be regarded as linear, then, for a minimum phase system (see, for instance, Machin, 1964, or Milsum, 1966), the phase corresponding to a straight line on the frequency response should be not only constant but expressed by  $(90 \alpha)^\circ$ . Fig. 5 shows a plot of  $\Phi_1$  against  $(90 \alpha)^\circ$  for all the preparations.

Clearly the points lie close to the line  $\Phi_1 = (90 \alpha)^\circ$  and a second linearity criterion is therefore satisfied. In the remainder of the paper the mean of

$\Phi_1$  and  $(90\alpha)^\circ$  is referred to as  $\Phi$ . The graph also shows the range of values of  $\Phi$  encountered (4–45°).

One would like to know whether not only the slopes of the lines fitted to the frequency response varied, but also whether their positions did. This was investigated in those preparations where the frequency responses were normalized by dividing by the static sensitivity and it was found that

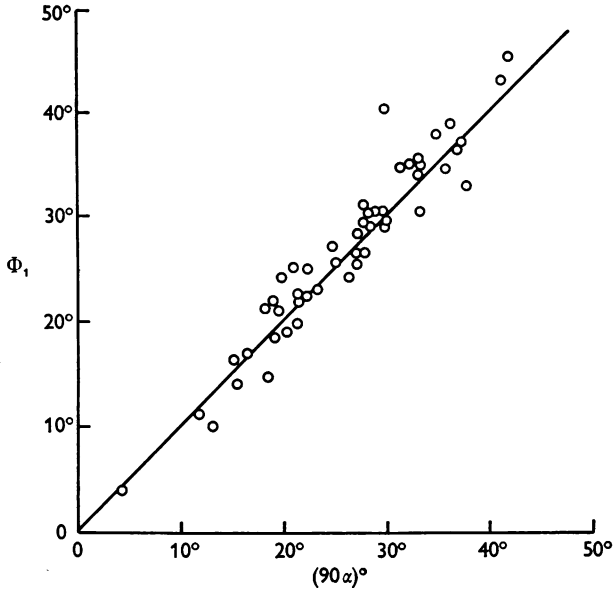


Fig. 5. Minimum phase behaviour for stretch frequencies up to 2 Hz.  $\alpha$  is the slope of straight lines fitted by eye to the frequency responses below 2 Hz, and  $\Phi_1$  is the mean measured phase over the same range. For a minimum phase system,  $\Phi_1 = (90\alpha)^\circ$ . The line represents this relationship.

a systematic variation existed. It was found that for eighteen out of twenty plots the frequency responses below 2 Hz were all compatible with a family of straight lines through a common intercept on the frequency axis, the value of that intercept being about 0.00018 Hz. An important illustration of this relationship is shown in Fig. 6. This shows that the values of amplification at 0.1 Hz plotted against  $\Phi$  fit tolerably well with the unique representation implied in the above common intercept model. This means that the value of amplification at 0.1 Hz may be taken as roughly representative of  $\Phi$ .

The good fit to such a constant intercept raises another possibility. Without negating any of the above paragraph it is possible that the observed relationship is an experimental artifact resulting from the arbitrary definition of the response as 'static' after about 20 min. However, the simplest explanation of this kind, that the

frequency response should continue as a straight line for all frequencies down to zero, i.e. a transfer function of the type  $ks^\alpha$  (Chapman & Smith, 1963), was not a good fit, particularly for high values of  $\Phi$  where the measured static responses were up to 2.5 times larger than predicted values calculated from the frequency responses. The calculations were done on the basis of the measured straight line frequency responses and the actual times used for measuring the static responses, on the assumption of a step change of length. The fact that the measured static responses were larger than

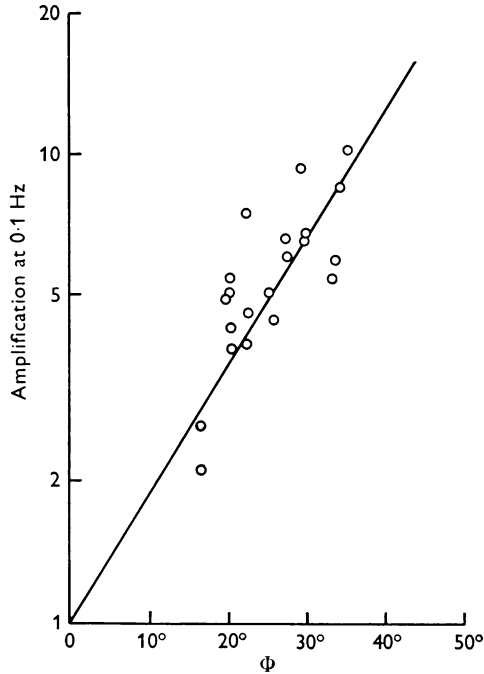


Fig. 6. Measurements from eleven different units showing the relationship between the amplification at 0.1 Hz and  $\Phi$ . The line represents what would be expected from straight line frequency responses with constant intercepts of 0.00018 Hz.

these values thus suggests that the constant intercept represents, if not a true static response, at least a partial levelling out of the frequency response around 0.0002 Hz, even if slower components of adaptation do also exist. Their presence is suspected because of the fact that most of the mean firing frequencies fell slightly during the 2 or 3 hr used for measuring the frequency response. However, other, extraneous factors could be responsible for this and it does not seem profitable to pursue these very slow events any further; very constant conditions and a complete history of the length of the spindle over many hours would be required for such a study.

The one preparation (two plots) excluded from the general analysis of the low frequency results was one whose values of amplification were consistently about 2.5 times larger than was general for the particular values of  $\Phi$ .

### *High frequencies*

Some of the variation between the responses of different preparations at frequencies above 2 Hz can be seen in Figs. 3 and 4. The basic feature was a peak, which was always present, with a maximum between 7 and 16 Hz, together with a rapid decrease in phase lead with frequency, the phase becoming a lag between 5 and 15 Hz. The variation in size and shape of the peak is discussed below in terms of the curves that can be fitted to the experimental points.

### *Curve fitting*

One type of curve that can be fitted to the frequency responses below 2 Hz, a set of straight lines, has already been described. Another type is fitted to the responses of Figs. 3 and 4. This type of curve corresponds to the type of analogue suggested by Kirkwood (1969) and could be fitted to all the frequency responses measured. In each case the phase measurements were in agreement with a minimum phase system represented by the curve fitted to the sensitivity plot. (The deviation above 20 Hz is discussed later.) The type of analogue considered consists of a number of phase lead elements connected in cascade. (Each element has the transfer function  $(1 + aTs)/(1 + Ts)$  where  $a$  is a constant,  $T$  a time constant and  $s$  the Laplace Transform variable.) It should be noted, however, that the accuracy of the experiments was not sufficient for much reliance to be put on the values chosen for corner frequencies, neither is it possible to choose between this representation and the straight line one on the evidence of these experiments alone. They are not necessarily different in kind; the straight line could represent merely a distributed form of the other. The straight line has attractions in its simplicity but there was some evidence of deviations from it. One example of this has already been mentioned when the static response was considered. Another comes from the phase measurements at 0.1 Hz, which were mostly (fifty out of seventy) lower than the corresponding values of  $\Phi$ , indicating consistent deviations from constant phase behaviour.

In order to fit the points in the high frequency range, it was found necessary to use a curve corresponding to a velocity sensitivity combined with a second-order lag [i.e. with the same terminology as above, a transfer function of the form  $(1 + aTs)/(1 + 2\zeta Ts + T^2s^2)$ ]. The continuous lines of Figs. 3 and 4 correspond to this. The value of the damping factor,  $\zeta$ , of the fitted curves was variable. This is the parameter that varies the sharpness of the peak of the frequency response, a sharp peak corresponding to a low value of  $\zeta$ . When  $\zeta < 0.707$  the system can be said to be resonant and an

overshoot in the response to a ramp will be apparent. The lowest value of  $\zeta$  fitted was 0.6 and the highest was 2.5.

The principal variable affecting  $\zeta$  appeared to be the mean firing rate.  $\zeta$  also correlated well with  $\Phi$ , which is not surprising since both the firing rate and  $\Phi$  were high at long muscle lengths where, in general,  $\zeta$  was low. However, the evidence for choosing firing rate as the important variable comes from incomplete observations in a few preparations where, at a constant length, the mean firing rate was decreasing. In these circumstances it was seen that both the sensitivity and phase at frequencies around 10 Hz also tended to decrease, whereas the sensitivity in the lower frequency range tended to stay more nearly constant. Where it did tend to fall, moreover, the phase usually stayed constant.

The phase measurements deviate from the solid line for the higher frequencies in Figs. 3 and 4. A variety of factors still within the domain of linear theory could explain the extra phase lag. For instance, in Fig. 3 a finite time delay of 2 msec fits nearly all the points (dashed line). Now, a finite time delay might be expected for conduction time but in these experiments with electrodes near the muscle, this should not be more than 0.5 msec. An alternative curve is obtained by adding a further resonance at 100 Hz with its own damping factor of 0.4 (dotted lines). This has the added recommendation that the line passes through the 100 Hz amplification point. Many other combinations of these types of curves together with simple first-order lags could be postulated but several measurements above 100 Hz would be required to decide between them. Fuortes & Hodgkin (1964) suggested a similar multistage lag system to explain a time delay in the response of *Limulus* ommatidia. All the frequency responses measured could be fitted in this way without needing to involve a time delay greater than 0.5 msec, but a single finite delay of between 0 and 4 msec could have been used instead (mean 1.5, s.d. 1.0, fifty-one observations).

#### *Variation of response with length*

It was observed in all eight preparations where the frequency response was measured at two lengths, that  $\Phi$  increased with increase in muscle length (e.g. Fig. 4) but systematic measurement of the frequency response at more than two lengths was prevented by the time over which any one preparation would give consistent responses. Instead, measurements at one frequency only (usually 0.1 Hz) were made at different lengths and these measurements can be taken as representative of the variation of  $\Phi$ , as described earlier.

The first measurements of this kind were made on multi-unit preparations and the results are shown in Fig. 7. It can be seen that not only are the results consistent in that all the points lie roughly on the same curve,

but also that a simple relationship fits the points, namely a linear increase in  $\Phi$  with length. The phase points may appear to be not such a good fit to this relationship, but it should be remembered that a very similar discrepancy was noted between the measured values of 0.1 Hz phase and  $\Phi$  from the variable frequency experiments.

In the case of the single units, however, the situation was not so consistent. Eight out of ten units showed a rise in 0.1 Hz amplification (and

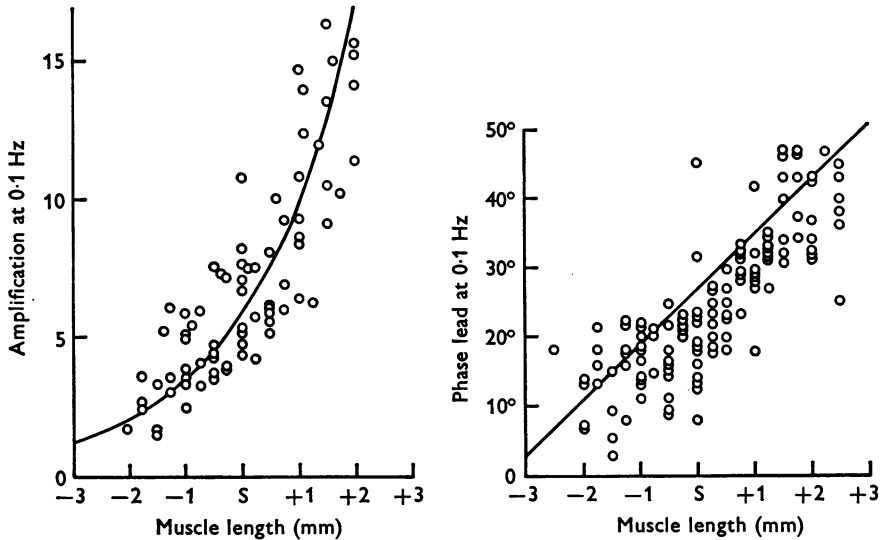


Fig. 7. Variation of response with muscle length from eleven multi-unit preparations. *S* is the length corresponding to the maximum static sensitivity. The two curves correspond to the constant phase model with a frequency response intercept of 0.00018 Hz and a linear variation of  $\Phi$  with length. The poor fit of the line on the phase plot is very similar to the observed differences between the 0.1 Hz phase and the measured values of  $\Phi$  from the frequency response plots.

therefore in  $\Phi$ ) with muscle length, though the rate of increase and the relationship of this to the static characteristic were variable. Three examples are given in Fig. 8. The frequency response of the unit which showed a decrease with length (*c* and *f* in Fig. 8) was also measured between 0.003 and 5 Hz, giving measurements in good agreement with the usual type of constant phase, constant intercept pattern. This unit was therefore not suspected of being unusual in any other way.

#### *Responses from cut-down spindles*

The positive slope of the frequency response and the corresponding phase lead are a measure of the dynamic response and consequent adaptation seen to transient stimuli. A popular theory to explain this phenomenon

(Matthews, 1931) has been that it arises from a difference in the mechanical properties of the polar and central regions of the intrafusal fibres. It follows from the theory that any alteration of the mechanical state of the polar parts of the spindle should affect the response of the spindle to stretch of the muscle. It was for this reason that the measurements were made on the spindles where the polar parts had been removed. It should be said that in these cut-down preparations there still remained a lot more

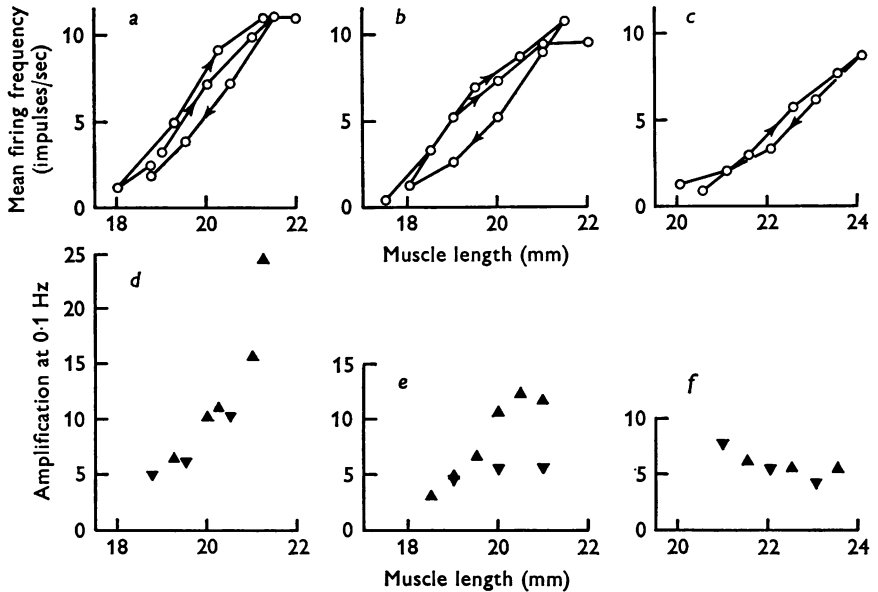


Fig. 8. Variation in response with muscle length from three single unit preparations. *a, b, c*: static characteristics of the three units; arrows show the order of taking readings. *d, e, f*: corresponding variation of amplification at 0.1 Hz (▲ length settings in ascending order, ▼ length settings in descending order). Dissection lengths of the three muscles were 23 mm, 21.5 mm and 24.5 mm respectively.

of the intrafusal fibres than the reticular zone, which is the region often assumed to have low viscosity (e.g. Houk, Cornew & Stark, 1966) and which is only of the order of 100  $\mu$  long (Katz, 1961). However, the transection and removal of 90% of the remainder might be expected to make some difference to the mechanical properties. Fig. 9 shows the frequency response of a spindle measured before and after this operation. This result seems clear enough; the shape of the frequency response, and hence the adaptive properties of the spindle, were unchanged.

There were, however, some differences between the responses before and after the operation. In particular the sensitivity and the mean firing rate



were often much more variable after than before the operation, the two varying apparently in proportion. This latter property was taken as an indication that the variation was due to some factor independent of those determining the shape of the frequency response, a surmise that was borne out by the fact that the phase measurements were repeatable in all preparations. The variation in sensitivity made it impossible to measure the sensitivity plot for five out of ten cases, but since in the other five and in all the undamaged spindles the sensitivity and phase plots corresponded,

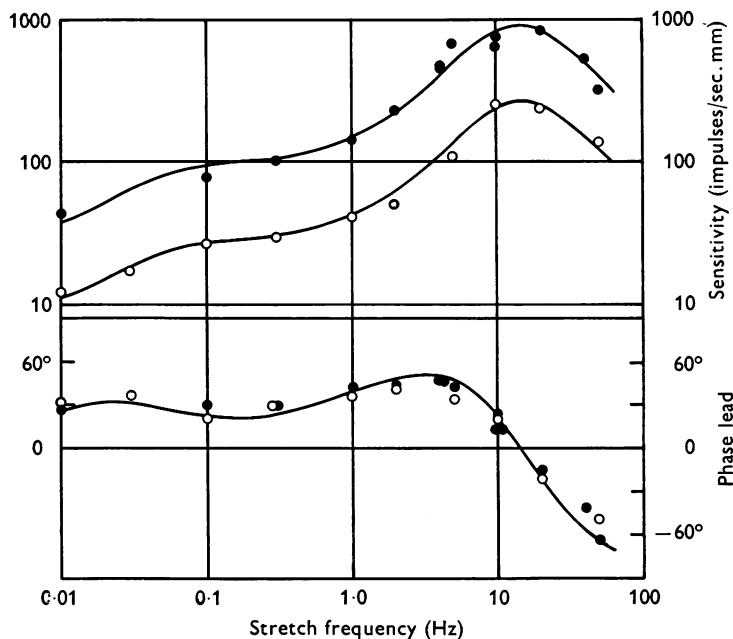


Fig. 9. Frequency response of a spindle unit before and after removal of the polar parts. Open circles: intact muscle, length 18.5 mm. Filled circles: cut-down muscle, length 3.6 mm. The curve on the phase plot corresponds to that of a minimum phase system represented by either of the curves on the sensitivity plot.

the phase plot alone could be taken as representative. Given that proviso, in nine out of ten cases the frequency response was the same shape before and after the operation. It also seemed to be the case that the variation of the response with length was also unaffected (Fig. 10) and, as a consequence, the particular value of  $\Phi$  was not necessarily the same before and after the operation. In cases like that shown in Fig. 9, however, where the length of the cut-down preparation was adjusted so as to give about the same mean firing rate as before the operation, the responses differed only

in the absolute values of the sensitivities. These were always higher in the cut-down preparations, as would be expected from their shorter lengths.

The one preparation that was excluded from the general result gave a normal, constant phase, frequency response in the undamaged state. However, when cut-down it gave approximately zero phase below 0.1 Hz but a phase around  $35^\circ$  at 1 and 2 Hz. It should be noted, though, that two of the intact preparations gave low frequency phase measurements which varied by almost as much as this (cf. Fig. 4).

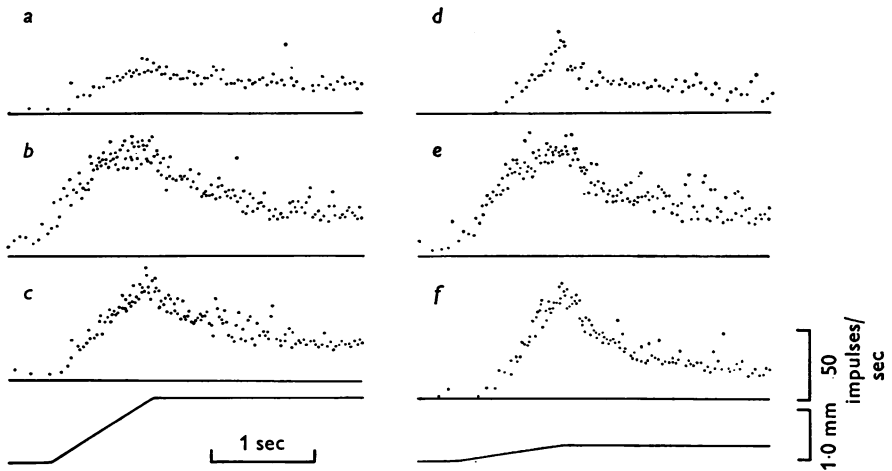


Fig. 10. *a, b, c*, response of an intact spindle to the ramp stretch shown below *c*. *d, e, f*, response of the same, but cut-down, spindle to the ramp stretch shown below *f*. *a*, muscle length 19.0 mm. *b*, 5 sec after a manual stretch (10 sec) to a length of 19.9 mm. *c* 1 min later at 19.9 mm. *d*, muscle length 2.3 mm. *e*, 7 sec after a manual stretch (10 sec) to a length of 2.6 mm. *f*, 1 min later at 2.6 mm. The calibrations apply to all records *a-f*. The variation in the response with time after the increase in background stretch was only seen rarely. It is of interest in this case because it occurred in both the intact and in the cut-down preparations. It was never seen in one without being present in the other. The effect is not discussed in the text.

#### *Comparison of sine and ramp data*

In order to test the validity of the frequency response representation beyond the restricted ranges of length, velocity and firing frequency for which it was measured, a number of preparations were also subjected to ramp stretches of one second duration and up to 2 mm in amplitude. For eighteen of the frequency responses, corresponding ramp responses were also recorded. Examples of the ramp responses are shown in Figs. 10, 11 and 12. In order to compare these with the data from the sinusoidal

stretches it was necessary to predict the theoretical ramp responses resulting from the measured frequency responses.

The first point to note in this respect is that neither the very low nor the high frequency components of the frequency response have much effect on the transient responses over the time scale used in many investigations, including this one. The straight line frequency response can thus be used as a good approximation to predict the responses to the ramps used in these experiments. If this representation is used then a transfer function of the type  $ks^\alpha$  (Chapman & Smith, 1963) may be assumed and the response to a ramp is given by  $y = Cvt^{1-\alpha}$ , where  $v$  is the velocity of the ramp,  $t$  the time after the start of the ramp,  $y$  the resulting change in firing frequency and  $C$  is a constant dependent on the static sensitivity,  $K$ , and on the value of  $\alpha$ ,

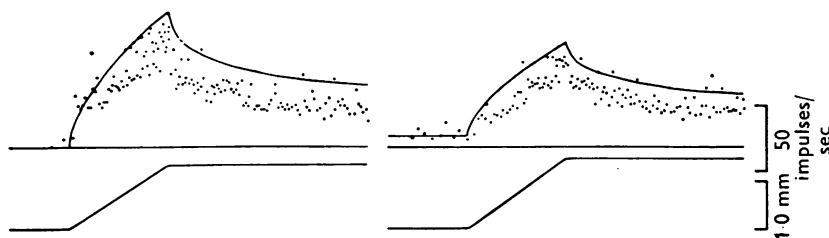


Fig. 11. Ramp responses from two different preparations. The curves represent the responses predicted from their frequency responses below 2 Hz.

the slope of the frequency response (see Appendix). If a common intercept of 0.00018 Hz on the frequency response is assumed, then for values of  $\alpha$  between 0 and 0.5,  $C$  varies approximately exponentially with  $\alpha$  from 1.0K to 33.7K, approximately doubling for each rise of 0.1 in  $\alpha$ . When this increase in  $C$  is coupled with the fact that for  $t < 1$ ,  $t^{1-\alpha}$  also increases with  $\alpha$ , it can be seen that a small increase in  $\alpha$  leads to a large increase in the dynamic response.

This increase in the dynamic response can be seen in Figs. 10 and 12, where an increase in  $\alpha$  (i.e. an increase in  $\Phi$ ) was brought about by increasing the mean length of the muscle. (The frequency response was not measured for these preparations at these particular lengths.)

Fig. 11 shows theoretical curves calculated as above fitted to two ramp responses from two other preparations. These are typical records and show features general to all the responses compared in this way. The measured responses are the same general shape as those predicted but are slightly smaller, in this case by a factor of 0.7–0.8. This factor was calculated for all the observed responses (81 observations) by measuring the ratio of the observed and expected values for the maximum dynamic peak. The mean

was 0.69 and the s.d. was 0.15. The factor was, in general, a little higher for the smaller amplitude ramps.

This is a fair measure of agreement, considering the limited ranges of length and velocity that were used to measure the frequency responses. It indicates only a small non-linearity that must be added to the description to extend by as much as a factor of five the range of firing frequencies over which the analysis is valid.

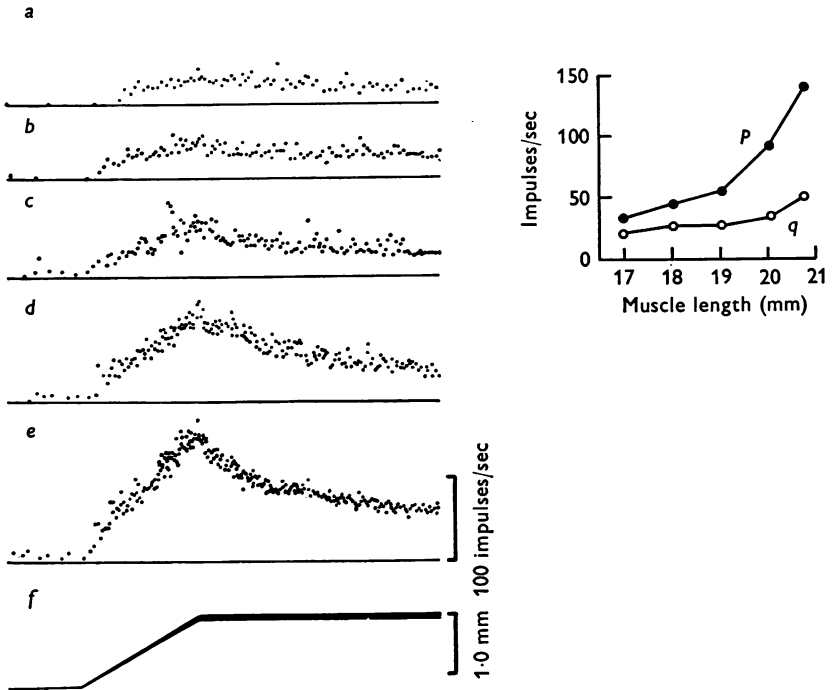


Fig. 12. The response of a unit to one second ramps at five different mean lengths. *a-e* responses to the ramps in *f* at mean muscle lengths of 17, 18, 19, 20 and 20.75 mm respectively. The dissection length for this muscle was 21 mm. *f* shows the ramps superimposed. (There was a slight variation in size, as can be seen.) The graph shows the maximum dynamic response, *p*, and the response two seconds later, *q*, plotted against mean muscle length. Note that  $p/q$ , as well as *p* and *q*, increases with muscle length.

The ramp responses demonstrated a similar dependence on the mean length of the preparation to that shown with the sinusoids. Fig. 12 shows the variation in ramp responses seen over a range of length of nearly 4 mm, which corresponds to over half of the physiological range. The variation in the maximum dynamic response, *p*, is typical of all the fourteen preparations where ramps were imposed at more than one length. The variation

in the size of the response 2 sec later,  $q$ , however, was more variable, although the majority of cases (nine) showed a significant increase in the ratio  $p/q$  with length, as does the example in Fig. 12. The increase in this ratio for most preparations is exactly what would be expected from the increase in  $\Phi$  with length for most preparations, which was demonstrated earlier.

#### DISCUSSION

It is of interest to compare the results of this study with those of other workers. Only one other frequency response study of frog spindles has been published (Houk *et al.* 1962), in which only two preparations were used and no checks on linearity were made. The measurements here must therefore be compared with the responses to the more usual types of transient stimuli. The fair correspondence between the sine and the ramp data in this study have already been described and the ramp responses are quite similar in general terms to those published in the past (e.g. Matthews & Westbury, 1965). Some details are different, e.g. there is little sign of the initial high frequency burst noted by Toyama (1966) or the pause after the end of the ramp seen by Shepherd & Ottoson (1965). It should be noted, however, that both these studies involved either long stretches or high velocities, exceeding the ranges used here, and also that the addition of a resonant component, such as was sometimes observed in this study, could explain a part of these phenomena.

The wide range of time constants implied in the constant slope frequency response also allows agreement with a wide range of authors, such as Toyama (1966), who fitted curves with a time constant of around 150 msec, Houk *et al.* (1962) (5 sec) and Van Leeuwen (1949), who observed adaptation over 3 or 4 min, though in this case to a constant load. Because of the long time allowed for adaptation in the present study the observed static firing frequencies were low (always below 18 impulses/sec) but were similar to those reported by Jahn (1968*a*) or Van Leeuwen (1949), both of whom held the stimulus constant for comparably long periods.

The variation in the responses with the mean length of the preparation is an important result and has not been described before for frog spindles, although some of the results of Ito (1969) and of Ottoson, McReynolds & Shepherd (1969) suggest a similar effect. The uniform variation over such a wide range of frequencies is believed to be unique for receptors. It should be noted that the lengths over which the variations were described here were all within the physiological range of passive spindles.

*Spindle mechanisms*

The results are of most interest when the implications for the mechanical theory of adaptation, that was first put forward by B. H. C. Matthews (1931) and was considered in more detail by P. B. C. Matthews (1964), are considered. Briefly, the theory suggests that the adaptive properties of the spindle arise because the mechanical time constant of the central sensory part of the spindle is shorter than that of the contractile polar parts. An extension of this theory that was put forward in the preliminary report of this work (Kirkwood, 1969) may first be considered. One of the suggestions put forward then was that the wide range of frequencies where the phase was leading could result from a range of mechanical time constants related to the complex tandem arrangement of spindles in this particular muscle. This was an attractive idea, because it also provided for some variation in the responses, those responses which showed dips in the phase plots being considered as representing spindles with a more simple structure. [Single spindles have been seen in this muscle but they are few in number (Barker & Cope, 1962). Moreover, the afferents can branch to innervate more than one capsule, so that the probability of recording from one totally single spindle is low.] The one ending that gave a different frequency response when cut down could then be considered as one which had been rendered single by the operation.

However, in order to accommodate the results of the other nine units whose frequency responses were essentially unchanged by the operation, one would have to assume that all the mechanical discontinuities responsible for generating the various time constants were all located within the 2 or 3 mm isolated. This is unlikely, so one is forced to conclude that the experiments with the cut-down spindles succeeded in disproving at least this extension of the mechanical theory. These experiments also provide evidence against the suggestion that some of the components of adaptation might result from length changes outside the spindle such as was suggested by Husmark & Ottoson (1971).

It is believed that the results from the cut-down spindles also provide evidence against the mechanical theory in general, although in this case it is not so conclusive. The deductions depend on the assumption that the operation should have altered the mechanical properties of the intrafusal fibres, or rather the properties of the remaining parts of them. There is evidence (Jahn, 1968*b*) that the elasticity of the central region, at least, is affected by similar damage to the intrafusal fibres. There are, in fact, three possible explanations for the insensitivity of the responses to the operation.

1. The mechanical theory of adaptation is correct and both the viscosity and elasticity of the polar parts are unaffected by the operation. (For this

to be the case, the mechanical properties of the polar parts of the intra-fusals would need to be completely uniform, so that the 10% remaining after the operation could represent what had been there before.)

2. The mechanical theory is correct and the operation affected the elasticity and viscosity of both the central and polar parts such that all the time constants were the same under all conditions as they had been before.

3. At the frequencies concerned, the relative viscosities of the central and polar parts of the spindle have very little influence on the response so that change in one of them has little effect. (Changes in elasticity are allowed for because absolute values of response amplitudes are not being considered.)

It is the author's opinion that the third of these is much the most likely.

It should be said that this evidence from cut-down spindles is not entirely new. Ottoson and his co-workers (Ottoson, 1961, and later papers) have worked on isolated spindles cut down to about 1 mm in length for some years, but they have not done the control experiment of showing that the responses that they record are the same as those of normal spindles. It is believed that the experiments described here go some way to providing that control and thus provide support for (i) the experiments which failed to demonstrate an overshoot in the extension of the central region of an isolated spindle when subjected to a step-like stretch (Ottoson & Shepherd, 1970) and (ii) those where the overshoot in tension and in the receptor potential were shown to be unrelated (Husmark & Ottoson, 1971). With frequencies as low as 0.001 Hz, the present results also cover a very much greater range of times than the 100 msec that has been investigated by Ottoson and his colleagues.

Some of the later interpretations of the mechanical theory are vulnerable to the evidence presented below. Interpretations of the effects of fusimotor stimulation on the response to stretch, such as those recorded by Matthews & Westbury (1965), are implicated, particularly if these interpretations are in terms of alterations of the mechanical properties of the intra-fusals at some distance from the capsule. Moreover, the present experiments also provide an alternative explanation for at least part of the effects seen in these conditions. Fig. 10 is relevant here. It shows that not only additional, steady stretch affects the response to a ramp but that also the same effect can be seen when the muscle is cut down. The different responses recorded by Matthews & Westbury (1965) could thus be said to result at least in part from the type or extent of stretch applied to the central, non-contractile part of the intra-fusals. Perhaps even the whole of the effects that they saw could also be explained in this way if the intra-fusals contraction could extend the centre by a greater amount than was

done in these experiments. The experimental stretches were restricted so as not to damage the preparation, but this need not apply to the more limited type of extension that could take place only within the spindle.

These experiments have little to say about the many other possible origins of the adaptive properties, except that the fact that the frequency response is a straight line for a wide range of values of  $\Phi$  suggests that one factor alone is responsible for determining the response in this frequency range. However, it should be noted that the experiments of Poppele & Bowman (1970) in the cat suggest the reverse, in that they observed a long time constant of adaptation (about 25 sec) only with their type B (primary) units and not with their secondaries.

One hint as to the cause of the resonance effects comes from the apparent dependence of  $\zeta$  on the mean firing frequency. In all cases of  $\zeta < 1.0$  the firing frequencies were between 6 and 14 impulses/sec. In other words, it may be that a certain degree of synchronization is taking place because the stretch frequency is near to the mean firing frequency. However, this must be a small effect superimposed on an already extant peak since preparations firing at, for instance, 5 impulses/sec did not show a corresponding peak in the frequency response at 5 Hz but only one at around 10–15 Hz, as for all the other preparations in these experiments.

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#### APPENDIX

##### *Derivation of ramp response of system with transfer function of the type $ks^\alpha$*

Notation	$y$	Firing frequency (impulses/sec)
	$t$	Time after start of ramp (sec)
	$v$	Velocity of ramp (mm/sec)
	$k$	Sensitivity at 1.0 radian/sec (impulses/sec.mm)
	$K$	Sensitivity at 0.00018 Hz (impulses/sec.mm) (0.00018 Hz = 0.001131 rad/sec)
	$Y$	Laplace Transform of $y$ ( $= \mathcal{L}[y]$ )
	$s$	Laplace Transform variable
	$\alpha$	Slope of frequency response

Laplace Transform of ramp  $= v/s^2$

therefore

$$Y = ks^\alpha(v/s^2) = vks^{\alpha-2}$$



Write

$$k = k'\Gamma(2-\alpha) = k'\int_0^\infty e^{-u}u^{1-\alpha}du,$$

therefore

$$Y = vk's^{\alpha-2}\int_0^\infty e^{-u}u^{1-\alpha}du.$$

Put  $u = ts$ , therefore  $du/dt = s$ ,

$$\begin{aligned} Y &= vk's^{\alpha-2}\int_0^\infty e^{-ts}t^{1-\alpha}s^{1-\alpha}sdt \\ &= vk'\int_0^\infty e^{-ts}t^{1-\alpha}dt \\ &= vk'\mathcal{L}[t^{1-\alpha}], \\ y &= vk't^{1-\alpha}. \end{aligned}$$

Therefore the response to a ramp is given by  $k'vt^{1-\alpha}$ , where

$$k' = \frac{k}{\Gamma(2-\alpha)} = \frac{K}{\Gamma(2-\alpha)0.001131^\alpha}.$$

Values for  $k'$  are tabulated below:

$\alpha$	$k'$
0	1.0K
0.1	2.05K
0.2	4.17K
0.3	8.47K
0.4	16.95K
0.5	33.7K

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