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CINEMATOGRAPHIC ANALYSIS OF CONTRACTILE EVENTS PRODUCED IN INTRAFUSAL MUSCLE FIBRES BY STIMULATION OF STATIC AND DYNAMIC FUSIMOTOR AXONS

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SUMMARY

1. Muscle spindles with an intact blood supply and uninterrupted connexions with ventral and dorsal spinal roots (Bessou & Pagès, 1967, 1972) have been prepared in cat's tenuissimus muscles with the aim of cinephotographically recording intrafusal movements induced by the stimulation of single static or dynamic γ axons; the time course of these movements and the morphological kind of activated intrafusal muscle fibres have been established.

2. Displacements of spindle guiding marks in the equatorial region elicited by stimulating single static γ axons are 4–20 times greater in amplitude than the ones elicited by stimulating dynamic γ axons at the same frequency.

3. The dynamic γ axons induced a contraction only in nuclear bag fibres which, in addition, never received any static γ innervation. The static γ axons evoked contractions either in nuclear bag fibres alone, or in nuclear chain fibres alone, or in both types of intrafusal fibres. Two thirds of static γ axons supplied nuclear bag fibres. For various reasons, one half only of static γ axons innervating nuclear bag fibres could be shown to simultaneously innervate nuclear chain fibres. Consequently, about one third of static γ axons supplied both nuclear bag fibres and nuclear chain fibres, but it is highly probable that this latter figure is an underestimate. One third of static γ axons produced contraction in nuclear chain fibres only. In this work, the distribution of fusimotor axons has been established in only one muscle spindle of the cluster of muscle spindles that each fusimotor axon is generally innervating.

4. Generally speaking, a static γ axon elicits contraction of several intrafusal fibres whereas a dynamic γ axon innervates only one intrafusal fibre and frequently only one pole of the fibre.

5. One third of static γ axons evoked contractions in nuclear chain fibres that seemed to involve the whole pole. The other static γ axons and all dynamic γ axons produced, in the intrafusal fibres that they supplied, one or several foci of localized contractions.

6. The nuclear chain fibres contract and relax faster than nuclear bag fibres. The contractions of nuclear bag fibres supplied by static γ axons are stronger and faster than those of nuclear bag fibres innervated by dynamic γ axons. Nearly all nuclear bag fibres innervated by static γ axons, like the nuclear chain fibres, show transient contractions at each pulse of a stimulation at low frequency (2–20/sec).

7. The results are discussed taking into account the available anatomical and physiological data on the muscle spindle. Their consequences with regard to intrafusal working are briefly considered.

INTRODUCTION

The intensive investigations of mammalian muscle spindles have led to the precise description of the anatomical structure of this complex sense organ (see Barker, 1974) and knowledge of the principal functional features of the sensory endings as well as of their control by the fusimotor system (see Matthews, 1972; Hunt, 1974). However, in many respects it is still difficult to relate the physiological findings to the structural characteristics of the muscle spindles, particularly with regard to the control of the sense endings by fusimotor axons.

The fusimotor axons have been differentiated into two functional types on the basis of their effects on the velocity sensitivity of the primary endings (Matthews, 1962). The stimulation of static axons decreases the dynamic responsiveness of the primary endings to ramp stretch while the stimulation of dynamic axons exerts the opposite effect. So far the internal working of the spindle during these actions is rather a matter of speculation and in this respect the spindle is a 'black box', indeed the spindle input pathways (fusimotor axons) are functionally defined only by the modifications that their activation induces in the sensory information conveyed in one of the spindle output pathways (group I A fibre connected to the primary ending). Since it is generally accepted that the fusimotor axons produce their effects on the sensory endings by eliciting a contraction of the intrafusal fibres, understanding the manner in which the fusimotor axons achieve their dynamic or static actions needs first more precise investigations of the contractile properties of the intrafusal muscle fibres.

Two indirect approaches of analysing of intrafusal muscle activity have been successively carried out. In the first one, sensory endings have been

used as a natural mechanical transducer to obtain some information on the mode of contraction of intrafusal muscle fibres activated by their motor axons. The superposition of responses of spindle sensory endings recorded with an instantaneous frequency-meter (Bessou, Laporte & Pagès, 1968*a*) has been the method employed to obtain as complete as possible information on the time course of underlying contractions. Such frequencygrams of cat spindle primary endings (Bessou, Laporte & Pagès, 1968*b*), of cat spindle secondary endings (Bessou & Pagès, 1969) and of rabbit spindle primary endings (Emonet-Dénand & Laporte, 1969) provided interesting data on the contractile phenomena produced in muscle spindles by stimulation of static and dynamic γ axons. However, owing to the structural complexity of the spindle and the lack of knowledge concerning the characteristics of the generator potential and of the spike production interposed between the contraction and the sensory ending response the interpretation of frequencygrams was limited.

The second indirect approach of analysing intrafusal muscle activity has been to record the intracellular responses of single intrafusal muscle fibres to stimulation of a single γ axon functionally identified as static or dynamic (Bessou & Pagès, 1972). One third of static γ axons elicited propagated action potentials in intrafusal muscle fibres, the remaining static γ axons and all dynamic γ axons evoked junction potentials. In this work the histological type of the intrafusal fibres from which the responses were recorded was not established. Further, the intrafusal muscle fibres, the membrane responses of which were recorded during fusimotor activation, have been marked by electrophoretic injection of fluorescent dye (Procion yellow) and identified taking into account morphological and ultrastructural characteristics (Barker, Bessou, Jankowska, Pagès & Stacey, 1972, 1975).

The present work has been performed with the aim of evaluating by the means of a direct approach the characteristics of the mechanical events produced in the different types of intrafusal muscle fibres by the stimulation of single static or dynamic γ axons. The movements of sarcomeres of intrafusal muscle fibres or of intrafusal structures have been observed under the light microscope and recorded by cinephotography. These observations have been made on muscle spindles of tenuissimus muscle of the cat. This muscle, according to an experimental method earlier described (Bessou & Pagès, 1967, 1972), allows us to prepare *in vivo* muscle spindles with an adequate blood supply and with uninterrupted innervation from the spinal roots.

A film entitled *Observations sur la motricité des fuseaux neuromusculaires* (Bessou and Pagès, 1973*a*), showing the preparation and illustrating the

main results has been produced. Preliminary reports on this work have been published (Bessou & Pagès, 1973*b*; Bessou & Pagès, 1973*c*; Bessou & Pagès, 1973*d*).

METHODS

Twenty experiments were carried out on adult cats under Nembutal anaesthesia (40 mg/kg given I.P. and followed by small amounts injected I.V. when necessary). In each experiment a single group I fibre connected to a muscle spindle located in the distal third of the tenuissimus muscle was prepared by splitting L 7 or S 1 dorsal roots. As large a number as possible of single fusimotor γ axons, generally 4–6, innervating this spindle were prepared by splitting L 7 and S 1 ventral roots. The single fusimotor axons were identified as static or dynamic by the effects that their repetitive stimulation at 100/sec exerted on responses of the primary ending to phasic stretch (Matthews, 1962). The muscle spindles, which were prepared following the method described in preceding papers (Bessou & Pagès, 1967, 1972), were examined by microscopy at rest and during short periods of repetitive stimulation of single fusimotor axons over a wide range of frequencies (2–110/sec).

The muscle spindle, sufficiently thin to allow light to pass through it, was observed under bright field illumination with white light supplied by a low voltage tungsten lamp (100 W). The beam of light whose intensity could be adjusted by a potentiometer was directed towards the condenser by a mirror. This condenser was stopped down to obtain the best contrast between different tissue components so that the striation pattern and the edges of the fibre were clearly visible. By using an ocular micrometer, the distance between the region observed and the equatorial reference point was measured as well as the diameter of the observed intrafusal muscle fibre. The fibre diameter was measured in the plane where, by focusing from the top to the bottom of the fibre, the width appeared the largest. Sometimes the plane of focus was differently set for observation and for recording movements.

Although the refractive index of the Ringer solution was not similar to that of the fibre contents, gross refraction effects due to the cylindrical shape of the fibre were not too important an obstacle to the examination of the fibre or even the sarcomere spacing of the fibre.

When it could be ascertained that the movements of the sarcomeres of the observed intrafusal muscle fibres were due to the contraction of this fibre, the best focusing plane was chosen and the movements of the sarcomeres recorded photographically (Kodak 4 ×) with a 16 mm reflex cine camera (Beaulieu) through a light microscope (Leitz) fitted with an oil-immersion objective (Leitz 100 ×, N.A. 1.30). The microscope image was projected on the film by a 10 × eye-piece. The film speed used was 25 frames/sec and the exposure time was 7·5 msec. The magnification of the image in the film plane was 125 ×. The microscope and the camera, respectively mounted on independent mechanical supports securing orthogonal displacements, were easily and precisely moved so that any point of the spindle could be examined and filmed.

Determination of the time course of any movement was made on the film. Each frame was projected upon a screen using an analysing projector. The enlargement of the frame on the screen was 96 ×. Simultaneously two bright arrows were projected normally upon the screen from two indicators travelling on an optical bench provided with a millimetric scale and aligned parallel to the screen. In this way any distance could be easily measured on the projected image. On the screen 12 mm represented 1 μm in the *in vivo* muscle spindle. The error of position determination of any structure projected on the screen depended upon the quality of the image but was

never greater than ± 3 mm, which is equal to ± 0·25 μm. In this manner, the time course of displacements of equatorially marked points elicited by the stimulation of single fusimotor axons has been measured as well as translation movements of sarcomeres of the activated muscle fibre, each time one of these sarcomeres was well individualized. In the same way, the time course of length changes of a sequence of 20–40 sarcomeres has been established when the muscle striations were clearly visible. In some fibres, when the striation pattern was obscured, some guiding mark coupled with fibre movements and situated in the region of maximum displacement was searched for and the time course of the movement of these marks were measured during fusimotor stimulation. At times the displacements of easily visible tips of intrafusal fibres have been plotted against time. Studies of the movement of a mark were not always possible because it was difficult sometimes to find an observable structure which remained without any deformation on all frames during the period of stimulation. Moreover the study of displacements of equatorial structures do not afford information concerning the contraction of intrafusal muscle fibres which is so precise as the measure of shortening of a sarcomere sequence.

RESULTS

Displacements of equatorial structures of muscle spindles elicited by fusimotor stimulation

Direct microscopic observations of equatorial structure displacements occurring within the spindle on stimulating single fusimotor axons have been performed. The stimulation of static γ axons and dynamic γ axons produces movements of different amplitude in the intrafusal muscle bundle.

The microphotographs of Pl. 1 illustrate this point. Pl. 1, fig. *A*, shows the central region of a spindle at rest. The oblong clear region represents the periaxial space limited by the capsule. The bundle of intrafusal muscle fibres, approximately 40 μm in width, crosses the periaxial space. According to anatomical descriptions (Boyd, 1962) it may be considered that the central region over 300 μm corresponds to the region supporting the primary ending. A nerve strand coming from the nerve of the tenuissimus muscle enters through the capsule, runs obliquely upward and to the right in the space and finally meets the bundle of intrafusal muscle fibres. This nerve branch has always been easily observed in all spindles studied. It fairly regularly reaches the muscle bundle in its equatorial region. This meeting point has been used as a reference point to accurately locate the position in the spindle poles of regions being viewed. During fusimotor stimulation the meeting point remains obvious and follows the movement of the intrafusal bundle which is a further reason why the meeting point has been used as a reference point for studying displacements of equatorial structures. The position of the reference point itself may be accurately determined in relation to some immobile structures lying outside the spindle. Two adipocytes have been used in this way in Pl. 1. A tangential line has been drawn on the left side of the adipocytes to make it easy to

see the displacement of the reference point during repetitive fusimotor stimulation. Pl. 1, figs. *B* and *C* show the displacement of the reference point elicited by one second of repetitive stimulation at 110/sec of two single static γ axons. The displacements towards the left in fig. *B* and towards the right in fig. *C* respectively measure 45 and 14 μm. Pl. 1, fig. *D*, has been taken 1 sec after the beginning of stimulation at 110/sec of a single dynamic γ axon; there is no obvious displacement of equatorial structures and the reference point stands still.

In all spindles observed the stimulation at frequencies higher than 50/sec of static γ axons always elicited displacements of the reference point which were easy to observe under microscopic magnification of less than 50, whatever the strength of action of the static axon on the frequency of firing of the primary ending. On the other hand, with the same range of microscopic magnification not the slightest displacement was visible on stimulating dynamic γ axons, even at high frequency.

These observations afford evidence that the mechanical effects of static fusimotor stimulation are quite different to the mechanical effects of dynamic fusimotor stimulation. The equatorial displacement induced by static axon activation was very useful in rapidly detecting a spindle in the tenuissimus muscle which had been previously located by mechanical stimulation of its primary ending.

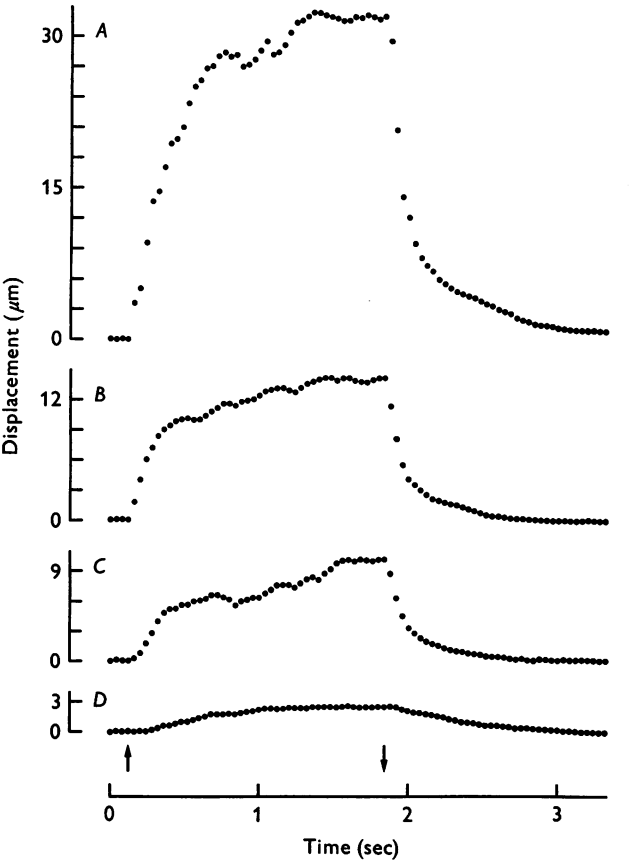
Time course of reference point displacements

Text-fig. 1 illustrates the time course of the equatorial movements in another muscle spindle during repetitive stimulation (100/sec) of three single static fusimotor axons (curves *A*, *B*, *C*) and of one dynamic fusimotor axon (curve *D*). Measurements were made using the reference point to build the curves *A*, *B*, *C* and a more subtle guiding mark located in the equatorial region of an intrafusal muscle fibre to build the curve *D*, since the reference point stays still during dynamic fusimotor stimulation. To increase the accuracy for building these curves measurements were made from cinematographic frames taken under microscopic observations at high magnification.

The time courses of the displacements elicited by stimulation of three single static γ axons are very similar in shape. It appears clearly in curves *A* and *B* that on the first frame following the beginning of the stimulation (upward arrow) displacement of the reference point occurs. On the contrary, in curve *C* the displacement is only discernible on the second frame. Thus the latency time is less than 40 msec in curves *A* and *B* but more than 40 msec in curve *C* since the film speed was 25 frames/sec.

During the contraction of the intrafusal bundle a fast motion phase is observed first then a slow motion phase. The mean velocity of the dis-

placement in curves *A*, *B* and *C* during the first 200 msec (i.e. 5 frames) of the fast phase of the movement is respectively 75, 40 and 25 μm/sec. Depending on which static γ axon is stimulated a maximum steady displacement is reached between 1·2 and 1·5 sec after the onset of the stimulation. Maximum displacement amplitudes are 32 μm (*A*), 14 μm (*B*) and 10 μm (*C*).



Text-fig. 1. Displacements from the resting position of equatorial guide marks of a muscle spindle produced by repetitive stimulation at 100/sec of three single static γ axons (*A*, *B*, *C*) and of one single dynamic γ axon (*D*). The equatorial 'reference point' has been used to build the curves *A*, *B* and *C*; another equatorial guiding mark has been used to build the curve *D* (see the text). The points represent measures of the displacement made on successive frames of a cine film. The rate of shooting the film was 25 frames/sec, 40 msec separating each point. In this and subsequent text-figures arrows indicate the onset and the end of the periods of stimulation. Conduction velocities: static γ axons, 34·2 m/sec (*A*), 36·4 m/sec (*B*), 35·2 m/sec (*C*); dynamic γ axon, 40 m/sec (*D*). Muscle spindle no. 10.

During the relaxation of the intrafusal muscle bundle the reference point comes back to its resting position first rapidly then more and more slowly. Most of the time the return phase lasts 1–1·5 sec and never exhibits an undershoot. Sometimes the reference point does not exactly return to the position held before stimulation. It stops 1–5 μm short of the original position.

On stimulating a dynamic fusimotor axon (curve *D*), in comparison with the preceding curves, striking differences in the time course of displacement may be observed. There is a far longer delay from the onset of the dynamic stimulation to the beginning of the movement (approximately 160 msec). The mean speed of outward displacement and of backward displacement is very low (approximately 2·5 μm/sec). The displacement is more uniform and its maximal amplitude is smaller (2·5 μm).

To sum up, Text-fig. 1 shows that the repetitive stimulation of static fusimotor axons evokes movements of the equatorial structures the amplitudes of which are greater than those of movements of the same region evoked by stimulation of dynamic fusimotor axons. In twenty spindles filmed the maximal amplitudes of movements elicited by repetitive stimulation of thirty-eight static γ axons were 4–20 times greater than those elicited by stimulation at the same frequency of eleven single dynamic γ axons.

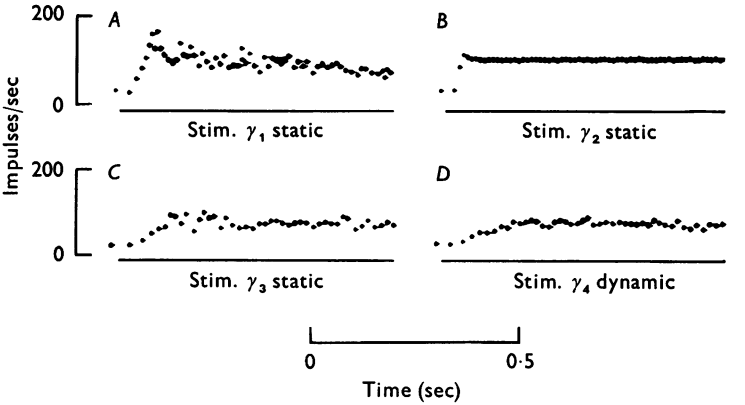
Displacement of the reference point results from an imbalance between the sum of contraction forces and the sum of visco-elastic forces. An interpretation of the observed differences in the amplitude of movement of the reference point evoked by stimulating static or dynamic axons may be differences in the value of the ratio of the contraction forces to the visco-elastic forces. This value could be high for the structures activated by static axons and low for structures activated by dynamic axons. The movement of the reference point towards a given pole indicates with certitude that this pole is supplied by the activated axon, but does not mean that the other pole is not supplied by the same axon; indeed unequal contractile forces may be induced in both poles by stimulation of a single fusimotor axon. Finally, due to the complex nature of the mechanical response from several fibres with properties varying from one to the other, movement of the reference point cannot give information on the contractile properties of individual intrafusal muscle fibres.

Microscopic observations and cinematographic recordings lead us to consider the displacement irregularities which are clearly visible particularly during the slow outward phase of the displacement elicited by static stimulation (see Text-fig. 1, curves *A*, *B*, *C*). These are the result of asynchronous activity in various parts of the intrafusal muscle bundle supplied by the stimulated axon and situated either in the same pole or

in the opposite pole. The asynchronous activity may be explained by some differences in (i) the conduction velocity of terminal branches of the stimulated axon; (ii) the capacity of transmission (facilitation, fatiguability) of excitation through the synaptic contacts; (iii) the extent and the velocity of the excitation conduction in the intrafusal muscle fibres.

Displacements of equatorial points and discharges of primary endings

In two experiments the primary ending discharges and the displacements of the reference point elicited by stimulating single fusimotor axons have been compared. The movements that are being compared with the discharges do not comprise the extension of the sensory region.



Text-fig. 2. Effects of repetitive stimulation at 100/sec of the three single static axons (γ_1, γ_2, γ_3) and of one dynamic axon (γ_4) on the instantaneous frequency discharge of a primary ending (muscle spindle no. 10 as in Text-fig. 1). Each point represents an action potential and its height above zero is the reciprocal of the time interval between it and the preceding point. Conduction velocities: static γ axons, 34·2 m/sec (γ_1), 36·4 m/sec (γ_2), 35·2 m/sec (γ_3); dynamic γ axon, 40 m/sec (γ_4).

The records of instantaneous frequency in Text-fig. 2 show responses of the primary ending of the same spindle as Text-fig. 1 to stimulation at 100/sec of each fusimotor axon. The discharge from the spindle has been recorded only during the first 660 msec of stimulation. After the onset of γ_1, γ_2, γ_3 and γ_4 stimulation the discharge reaches the maximum frequency respectively in 46, 31, 109 and 109 msec. The mean frequency of firing after 0·5 sec of stimulation is respectively 76/sec (*A*), 106/sec (*B*), 61/sec (*C* and *D*). The primary ending discharge exhibits some adaptation in the record *A*. In record *B* each stimulus evokes one afferent spike (driving). Some irregularities in the firing rate are noticeable chiefly in record *A* and

in records *C* and *D*; they progressively decrease and are small 0·5 sec after the start of stimulation.

The discharges of the primary ending (Text-fig. 2) have not been recorded at the same moment as the equatorial displacements (Text-fig. 1), but they can be correlated because in this experiment mechanical and, in particular, sensory responses stay constant over a long period of time. By comparing the records *A*, *B*, *C*, *D* of Text-fig. 2 to the curves *A*, *B*, *C*, *D* of Text-fig. 1 during the same period of stimulation it clearly appears that the rising phases of instantaneous frequency (Text-fig. 2: *A*, 46 msec; *B*, 31 msec; *C*, 109 msec; *D*, 109 msec) always last less time than the motion phases of displacements (Text-fig. 1, approximately: *A*, 1·2 sec; *B*, 1·2 sec; *C*, 1·4 sec; *D*, 1·5 sec) and that there is no clear relation between the amplitude of the plateau of primary ending discharge and the maximal amplitude of the displacement. Moreover some features are particularly striking, for instance: the frequency irregularities found in record *A* of Text-fig. 2 are not at all explained by the initial part of curve *A* of Text-fig. 1; the frequency regularity found in record *B* of Text-fig. 2 has no mechanical support at all in curve *B* of Text-fig. 1. Finally, frequency records which have similar time course and are quantitatively very close (records *C* and *D* of Text-fig. 2) correspond with very different movement of the equatorial region (curves *C* and *D* of Text-fig. 1). The equatorial reference point is always moving 1 sec after the onset of the fusimotor stimulation while the discharge of the primary ending at this point has been stable for a considerable time.

Movements of sarcomeres of intrafusal muscle fibres elicited by stimulation of fusimotor axons

As often as not the intrafusal fibres of an *in vivo* muscle spindle cannot be identified as nuclear bag or nuclear chain fibres by observing their nuclear arrangement in the equatorial region. Indeed in most cases the nuclei are masked by branches from the equatorial nerve. In this study the classification of intrafusal fibres was based on two criteria: length and diameter of fibres.

In histological preparations of muscle spindles from cat tenuissimus muscle the juxta-equatorial diameters for the two types of intrafusal fibres are 12–23 μm for bag fibres and 4–13 μm for chain fibres (Boyd, 1962). Since there is extensive overlap in the diameters of the two populations, fibres that were larger than 17 μm have been considered as nuclear bag fibres and those smaller than 12 μm as nuclear chain fibres. The diameter of an intrafusal muscle fibre was measured as far from the equatorial region as possible to avoid errors resulting from the slight decrease in diameter

usually observed in the juxta-equatorial region and at some distance from the extremity where the fibres taper over some length (this particularly applies to nuclear bag fibres).

The lengths of intrafusal fibres in histological preparations of muscle spindles from cat tenuissimus muscle are 4–13 mm for bag fibres and 2–6 mm for chain fibres (Boyd, 1962). Consequently the thick fibres that extend well beyond the presumed limits of the capsule have been considered as nuclear bag fibres and the thin fibres, the course of which was almost entirely intracapsular, have been considered as nuclear chain fibres.

Usually, chain fibres are far easier to observe than bag fibres. The course of bag fibres is rectilinear while that of chain fibres is sinuous because the fibres twist between themselves. The chain fibre striations are hardly visible owing to the frequency of super-imposition of muscle fibres and nervous branches that run over the whole length of the fibres. The bag fibres and their striations were easily visible particularly in the polar regions. In these regions the spindle capsule thins down and finally disappears, the number of intrafusal fibres decreases and the intrafusal fibres tend to spread out and pursue individual courses.

Patterns of sarcomere movements

Repetitive stimulation of fusimotor axons evokes different patterns of movements that could be summarized as two types, localized movements of convergence of sarcomeres and movements of translation of sarcomeres.

Localized movements of convergence. Looking at a fibre under the microscope, one observes a shortening of the sarcomeres in patches within the field of view. The contracted zone of the fibre is made up of a variable number of sarcomeres. The sarcomeres on both sides of the localized contraction shift, converging towards the centre of the contracting region. At the level of this stationary contraction node, some swelling of the fibre may sometimes be observed. It was always evident that no mechanical coupling with any neighbouring muscle fibre which might itself eventually contract could induce such a movement. So the movements of convergence of sarcomeres provides good evidence that the intrafusal muscle fibre observed is supplied by the stimulated fusimotor axon.

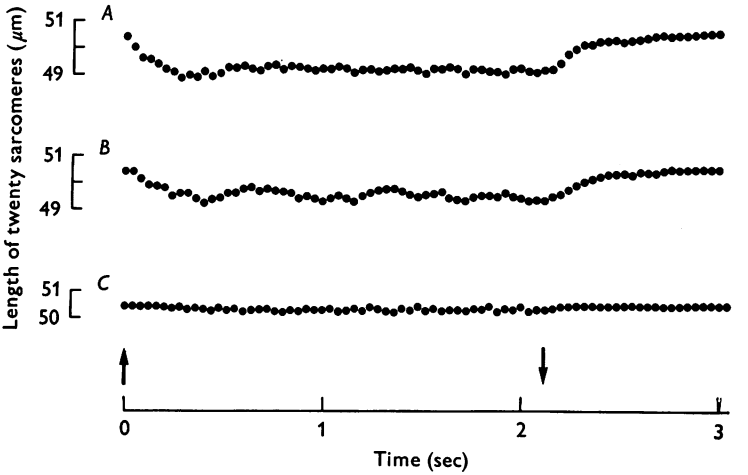
Pl. 2 illustrates this kind of sarcomere movement evoked by stimulation of a static γ axon in a nuclear bag fibre the diameter of which was 26 μm. Thirty-six sarcomeres are clearly visible at rest (Pl. 2, fig. *A*) and during the movements of convergence (Pl. 2, figs. *B* and *C*). In this case the sarcomeres are displaced in opposite directions towards an immobile region (white triangle). At rest (Pl. 2, fig. *A*) from the white arrow on both sides of each frame eighteen sarcomeres may be numbered. The length of the eighteen sarcomeres at the right side is 44·2 μm and at the left side

45·1 μm; the mean sarcomere length is 2·47 μm. After 200 msec of stimulation of a static γ axon at the frequency of 90/sec (Pl. 2, fig. *B*) the shortening of the sarcomeres on the left side is greater than on the right (respectively 1·9 and 0·9 μm) so that both fibre segments become 43·2 μm long; the mean sarcomeres length is 2·40 μm. Full shortening of the sarcomeres was reached 1 sec after the beginning of stimulation (Pl. 2, fig. *C*); the left and right eighteen sarcomeres have the same length (41·2 μm). The maximum shortening of the thirty-six sarcomeres is 6·8 μm (i.e. 7·6 % of rest length); the mean sarcomere length is 2·28 μm.

Movement of translation of sarcomeres. When sarcomeres of an intrafusal fibre were moving during fusimotor stimulation, the pattern of convergent movement previously described was not always observed, probably because the part(s) of the fibre where such a pattern occurred were hidden by some nervous, vascular or muscular structure overlying the fibres. It was possible in these cases to observe unidirectional shifting of striations. This pattern is called movement of translation of sarcomeres. Changes of striation spacing were not usually discernible, thereby direct evidence of contraction was missed. Moreover, when movements were eventually observed in several intrafusal fibres the shift of the striation of a given fibre did not necessarily indicate that this fibre was supplied by the stimulated axon. Because the intrafusal muscle fibres lie parallel to each other, mechanical interactions arising from this arrangement could well explain any translation movements. However, in some circumstances the translation movement of sarcomeres might be considered as indirect evidence of the contraction of a fibre and consequently of its innervation by the stimulated axon. To ascertain this fact, the main criteria were the immobility of neighbouring intrafusal fibres or if the immobility of the neighbouring fibres was not absolute, the smaller amplitude of their movements. Most of the time, by adjusting the stimulation rate of the fusimotor axon it was possible to get evidence of the contraction of at least one fibre among several shifting fibres. Generally it was easier to be confident that an intrafusal fibre was innervated by a fusimotor axon: (i) when the sarcomeres of one pole of this fibre were moving towards the polar extremity rather than towards the equatorial region; indeed a shifting towards the equatorial region of the sarcomeres of a spindle pole might be a movement transmitted through the spindle equator and due to the contraction of the other pole of the same fibre; (ii) when the intrafusal fibre was a nuclear bag fibre rather than a nuclear chain fibre because the bundle of nuclear chain fibres often move as a unit on stimulating a static γ axon, whatever the rate of the stimulation (a similar observation has been related by Boyd, 1966; Boyd & Ward, 1975).

Whatever the movement pattern, measurements were performed to

estimate the time course of the intrafusal contraction and to establish whether physiological differences occur in the mechanical process of the contraction firstly of nuclear bag and of nuclear chain intrafusal muscle fibres, secondly of the intrafusal fibres innervated by dynamic or static γ axons.



Text-fig. 3. Changes in the length of twenty successive sarcomeres of a nuclear bag fibre produced by repetitive stimulation of a single dynamic γ axon. The sarcomeres were located in the middle of a local contraction 3·95 mm distant from the equatorial reference point. Fusimotor stimulation frequencies: *A*, 110/sec; *B*, 65/sec; *C*, 45/sec. Conduction velocity of the dynamic γ axon: 40 m/sec. Width of the nuclear bag fibre at local contraction site: 21 μm. Muscle spindle no. 10.

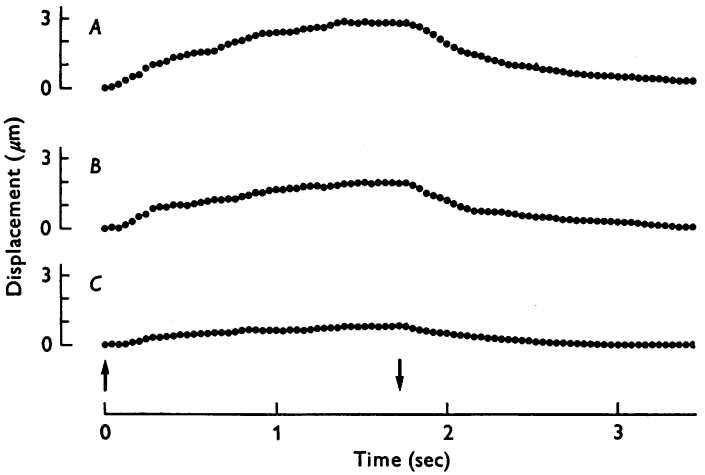
Movements in bag fibres elicited by stimulation of dynamic γ axons

Localized movements of convergence of sarcomeres. The length of twenty sarcomeres of a bag fibre in a region of movement of convergence has been measured during repetitive stimulation of a dynamic γ axon (Text-fig. 3).

Curve *A* (stimulation frequency: 110/sec) shows that the maximum shortening (1·5 μm) is reached within 300 msec. The steady state of shortening is reached after 500 msec. In the preceding few milliseconds there is a small yielding of sarcomeres on which it is difficult to comment owing to the limited precision of the measurements. Curve *B* shows that at a relatively high rate of stimulation (65/sec) there is a latency time of more than 40 msec before the beginning of mechanical events; the mean steady shortening is 1 μm, i.e. 2 % of the resting length. Finally in curve *C* dynamic fusimotor stimulation at 45/sec elicits an imperceptible movement; it is evident that any transient shortening cannot be measured following each individual stimulus.

Seven foci of localized points of convergence of sarcomeres evoked by stimulation of five dynamic γ axons have been observed in five nuclear bag fibres belonging to different muscle spindles. Two foci of convergent movements have been seen in each of two bag fibres during dynamic fusimotor stimulation.

Movements of translation of sarcomeres. The curves *A*, *B*, and *C* of Text-fig. 4 illustrate the time course of the displacement of a sarcomere of a nuclear bag fibre when stimulating a dynamic γ axon at respectively 110/sec, 77/sec and 44/sec.



Text-fig. 4. Displacements of a striation of a nuclear bag fibre produced by repetitive stimulation of a single dynamic γ axon. The striation was located in the distal pole, 1·45 mm distant from the equatorial reference point. The striation moved towards the end of the fibre pole. All other intrafusal fibres were motionless during the dynamic fusimotor stimulation. Fusimotor stimulation frequencies: *A*, 110/sec; *B*, 75/sec; *C*, 45/sec. Conduction velocity of the dynamic γ axon: 36·1 m/sec. Width of the nuclear bag fibre in the observed region: 20 μm. Muscle spindle no. 13.

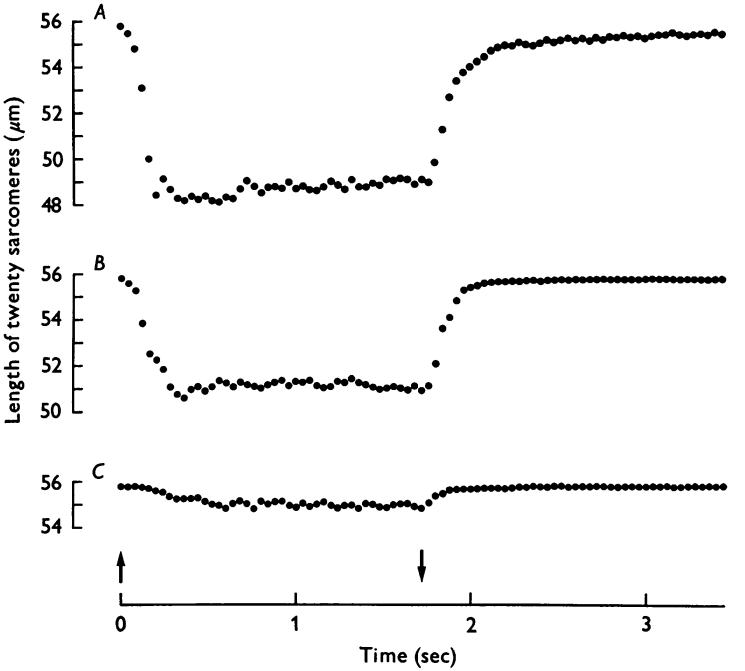
Between the onset of stimulation and the beginning of the shift of sarcomeres there is a latency time, the duration of which increases as the stimulation frequency decreases. The changes of the slope of the curves indicate that the displacement velocity varies during the stimulation. Whatever the frequency of stimulation, stable displacements are only observed after 1·25 sec of stimulation. At the end of stimulation the sarcomere slowly comes back to its resting position. This phase continues for more than 1·75 sec in *A*, 1·75 sec in *B* and 1·25 sec in *C*.

In this work thirteen translations of sarcomeres produced by stimulation

of six dynamic γ axons in six nuclear bag fibres of different muscle spindles have been observed and recorded on film.

Movements in bag fibres elicited by stimulation of static γ axons

Localized movements of convergence of sarcomeres. The time courses of shortening of twenty sarcomeres of a nuclear bag fibre during stimulation of a static γ axon at frequencies of 65/sec, 45/sec and 21/sec are respectively illustrated by the curves *A*, *B* and *C* of Text-fig. 5. In a general manner



Text-fig. 5. Changes in the length of twenty successive sarcomeres of a nuclear bag fibre produced by repetitive stimulation of a single static γ axon. The sarcomeres were located in the proximal pole, in the middle of a local contraction 1·05 mm distant from the equatorial reference point. Fusimotor stimulation frequencies: *A*, 65/sec; *B*, 45/sec; *C*, 21/sec. Conduction velocity of the static γ axon: 34·1 m/sec. Width of the nuclear bag fibre at local contraction site: 22 μm. Muscle spindle no. 16.

the shortenings elicited by static fusimotor stimulation have velocities 4–5 times faster and amplitudes 7–15 times greater than the shortenings elicited by dynamic fusimotor stimulation at the same frequencies. These differences appear clearly on respectively comparing the curves *A* and *B* in Text-fig. 5 with the curves *B* and *C* in Text-fig. 3; these curves result from stimulation at 65/sec and 45/sec of respectively a static γ axon

(Text-fig. 5) and a dynamic γ axon (Text-fig. 3). Curve C in Text-fig. 5 shows that static fusimotor stimulation at 21/sec evokes a maximum shortening of the same magnitude (1 μm) as that evoked by dynamic fusimotor stimulation at 65/sec (Text-fig. 3B). At a rate of stimulation lower than 10/sec the static γ axon did not evoke, in this experiment, any measurable shortening of the twenty sarcomeres (not illustrated in Text-fig. 5).

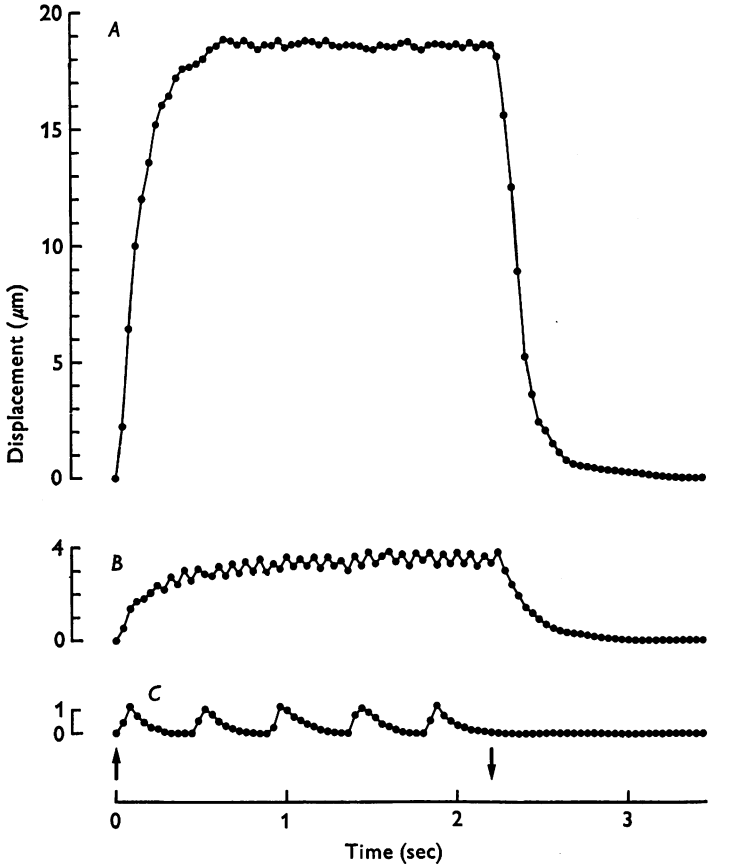
Periodic fluctuations of the length of the sarcomeres, the frequency of which are unrelated to the stimulation frequency, are visible on the plateau of the curve of shortening. These fluctuations are easy to observe not only on projecting the film but also on direct microscopic observation. The contracting segment of an intrafusal fibre looks like a concertina rhythmically expanded and compressed. Oscillations have been described during muscle contraction; Goldspink, Larson & Davies (1970) have described fluctuations in sarcomere length in the chick anterior and posterior latissimus dorsi muscles during isometric contraction; Speidel (1939) has reported a characteristic jelly-like quivering that accompanies the twitching of fibres in the tail of the living tadpole. However, it cannot be established to what extent the oscillations observed in our experiments may be compared with the preceding ones.

Fifteen foci of localized movements of convergence of sarcomeres elicited by stimulation of twelve static γ axons have been observed in eleven nuclear bag fibres belonging to ten spindles. In these observations, three static γ axons elicited more than one focus of local contraction in the same bag fibre, two static γ axons in two spindles were innervating the same bag fibre, and one static γ axon was connected to two bag fibres in an especially big spindle.

Movement of translation of sarcomeres. The time courses of displacements of a striation of a bag fibre elicited by repetitive stimulation of a static γ axon are illustrated in Text-fig. 6. In curve A the static axon was stimulated at the rate of 33/sec. The speed of the displacement which is very rapid during the first 240 msec (approximately 60 μm/sec) later becomes rather slow. The greatest longitudinal displacement (18·5 μm) is reached 750 msec after the onset of stimulation. No overshoot is visible at the end of the dynamic phase of displacement. Following the removal of stimulation the striation comes back towards its resting position in 1 sec, more than two thirds of the backward displacement being completed during the first 160 msec of relaxation.

The curve B illustrates the sarcomere movement when stimulating the fusimotor axon at 12·5/sec. The oscillatory character of the response clearly appears on the plateau of the curve. The oscillation frequency is the same as the stimulation frequency. As the film speed (25 frames/sec)

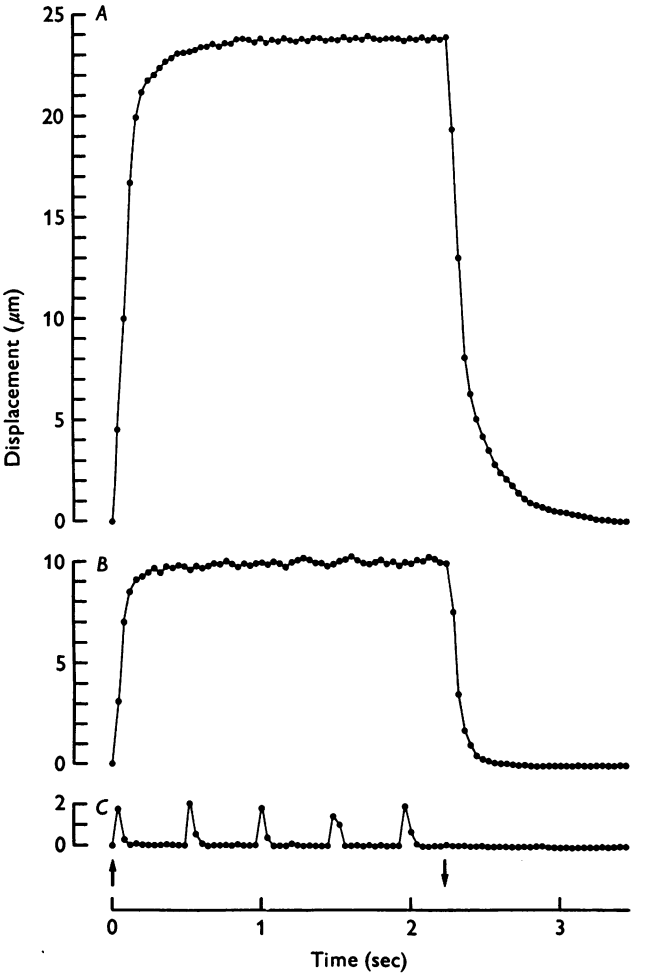
was twice the frequency of stimulation two measurements only are made at approximately the same moment of the twitch-like contraction elicited by each stimulus. The transient displacement induced by a single stimulus applied to the static γ axon can be analysed with a very low rate of stimulation (2·2/sec) as illustrated in record *C*. The maximum sarcomere displace-



Text-fig. 6. Displacements of a striation of a nuclear bag fibre produced by repetitive stimulation of a single static γ axon. The striation was located in the distal pole 0·66 mm distant from the equatorial reference point. The striation displacements were towards the end of the fibre pole. Two foci of local contraction were seen in this pole respectively 1·45 and 2·60 mm distant from the equatorial reference point. In addition the static γ axon made three nuclear chain fibres contract. No mechanical interactions arising from the parallel arrangement of the two kinds of intrafusal fibre, which were simultaneously contracting, have been found. Fusimotor stimulation frequencies: *A*, 33/sec; *B*, 12·5/sec; *C*, 2·2/sec. Conduction velocity of the static γ axon: 39·0 m/sec. Width of the nuclear bag fibre in the observed juxta-equatorial region: 18 μm. Muscle spindle no. 14.

ment of a twitch-like event is a little more than 1 μm. The 'contraction' and 'relaxation' phases of displacement respectively last at the most 80 msec and 200 msec.

Thirty-two instances of translation movement of sarcomeres evoked by stimulation of twenty-six static γ axons in twenty-one nuclear bag fibres



Text-fig. 7. Displacements of an extremity of a nuclear chain fibre produced by repetitive stimulation of a static γ axon. The axon supplied the fibre to the exclusion of any other. The length of the observed pole was 1·05 mm. The shift of the polar extremity was towards the equatorial region. The pole was free to move because the polar end was connected by small amount of slack connective tissue to surrounding perimysium. Fusimotor stimulation frequencies: *A*, 55/sec; *B*, 33/sec; *C*, 2·1/sec. Conduction velocity of the static γ axon: 34·2 m/sec. Width of the nuclear chain fibre at its widest polar region: 8 μm. Muscle spindle no. 13.

of eighteen muscle spindles have been recorded. In three muscle spindles two bag fibres were innervated by static γ axons. In five muscle spindles one bag fibre was innervated by two static γ axons.

Movements in chain fibres elicited by stimulation of static γ axons

In the poles of muscle spindles stimulation of certain static γ axons produced a simultaneous contraction of the poles of several chain fibres. The contraction was fast, strong and involved the full length of the activated poles. About one third of static γ axons elicit this type of contraction.

Localized movements of convergence of sarcomeres have been seen in chain fibres but defocusing of striation pattern during the contraction occurred frequently and made the microcinematography analysis very difficult. It is somewhat easier to film translation movements of striations or of the extremities of chain fibres. Fourteen of this kind of movement of nuclear chain fibres have been recorded.

The Text-fig. 7 shows the time course of the shift of the end of a chain fibre when stimulating a static γ axon. The polar extremity of the fibre was free to move because of the small amount of slack connective tissue connecting it to surrounding perimysium. Curve *A* was obtained with a stimulation frequency of 55/sec. In the first 200 msec of the contraction the speed of the displacement is very rapid (105 μm/sec). The steady displacement (24 μm) is reached after 800 msec of stimulation. The displacement towards the resting position, when repetitive stimulation is stopped, lasts a little more than 1 sec with an initial, very rapid phase followed by a slow one, with two thirds of recovery being complete 120 msec after the onset of the relaxation phase. At a stimulation frequency of 33/sec (curve *B*) the shape of the curve is similar to the preceding one but maximum displacement only reaches 10 μm. On the plateau of the curve some irregularities are visible. They are unrelated to the stimulation rate of the static axon. The curve *C* shows that each stimulus (stimulation rate 2·1/sec) produces a displacement of approximately 1·8 μm. The twitch-like event lasts approximately 120 msec with a 'time to peak contraction' of at least 40 msec, and a 'relaxation time' of less than 80 msec.

We may summarize as follows. (i) The contraction elicited in the nuclear bag fibres differs in its characteristics according to the functional type of fusimotor axons innervating these fibres. The contractions produced by the dynamic γ axons are 'slow' and small; the individual components are never discernible even at a very low rate of repetitive stimulation. On the contrary, contractions evoked by the static γ axons are 'fast' and large; the individual components may be distinct at low frequencies of stimulation. (ii) The contractions elicited by stimulation of static γ axons have

some different features according to the anatomical type of intrafusal muscle fibre being supplied. The contractions in the nuclear chain fibres are faster than the contractions in the nuclear bag fibres and they may frequently be elicited by a single stimulus applied to a single static axon.

Distribution of fusimotor γ axons to intrafusal muscle fibres

Since by visual examination of *in vivo* spindles it is possible to ascertain the morphological type of some intrafusal muscle fibres as well as the presence of contractions elicited by fusimotor stimulation in some of these fibres, the kind of intrafusal muscle fibre on which some nerve terminals of a γ axon are ending can be established.

The distribution of forty-nine fusimotor axons in twenty-one muscle spindles could be established (Table 1).

TABLE 1. Distribution of forty-nine fusimotor γ axons*

	38 static	11 dynamic
To nuclear chain fibres only	12 (about 1/3)	—
To nuclear bag fibres only	3 (underrated)	11
To both nuclear bag fibres and nuclear chain fibres	11 (underrated)	—
To nuclear bag fibres, but a possible distribution to nuclear chain fibres has not been determined	12	—

* This distribution was established by stimulation of *single* fusimotor γ axons and by observing the contractions of intrafusal fibres, produced by fusimotor stimulation, in only one muscle spindle of the cluster of muscle spindles that each fusimotor axon is generally innervating.

The stimulation of eleven dynamic γ axons has invariably produced the contraction solely of nuclear bag fibres. Thus, dynamic γ axons selectively supply nuclear bag muscle fibres.

Twenty-six static γ axons, i.e. approximately two thirds of the static γ axon population which was prepared, evoked contraction of nuclear bag fibres; for three of these axons it was confirmed that the bag fibres were the only activated intrafusal fibres; eleven of these axons produced contraction in both nuclear bag fibres and nuclear chain fibres; for the twelve remaining static γ axons the search for contingent contractions in nuclear chain fibres in addition to bag fibre contraction has not been or could not be carried out. Consequently the number of static γ axons innervating nuclear bag fibres only and the number of static γ axons simultaneously innervating nuclear bag and nuclear chain fibres are underrated. It can be estimated that more than one third of static γ axons are simultaneously distributed to both types of intrafusal muscle fibres.

These observations have also shown that static γ axons in general innervate several intrafusal fibres (nuclear chain and/or nuclear bag fibres) whereas dynamic γ axons almost invariably innervate only one nuclear bag intrafusal fibre and frequently only one pole of this fibre but with occasionally several foci.

Sometimes terminal branches of two or three static γ axons concentrate on the same bag fibre or on the same chain fibre. The bag fibres were found to be innervated either by dynamic or by static γ axons and a bag fibre supplied by both functional types of fusimotor γ axon was never observed.

In almost all muscle spindles which were observed mostly one, rarely two, static γ axons were found innervating both spindle poles.

Generally, static γ axons which simultaneously innervated the two types of intrafusal fibre supplied one sole bag fibre and one to four chain fibres. In such a case when a dynamic γ axon supplying the muscle spindle was prepared that axon was found to innervate the other bag fibre. Indeed, the muscle spindles of the tenuissimus muscle frequently contain two bag fibres.

The preparation used allowed only a qualitative study of fusimotor axon distribution focused on only one muscle spindle but the fact that one fusimotor γ axon may supply several spindles has not been overlooked. The visual observations of *in vivo* spindles do not afford the means of a quantitative analysis of the distribution of fusimotor axons for the following reasons: (i) both poles were not always examined with the same accuracy either because they were not always equally dissected or because the time during which the spindle or the fusimotor axons remained in good condition was limited; (ii) a weak focal contraction of the type that occurs in bag fibres may be missed if chain fibres are strongly contracting simultaneously in the same pole; (iii) the bag fibres were more extensively observed than chain fibres because our initial purpose was to know whether or not they were exclusively innervated by dynamic axons; (iv) in some instances, looking at chain fibres, several observations could be made on the same fibre especially if the regions observed were on different poles.

DISCUSSION

Importance of muscle spindle blood supply

Direct observation of movements in the muscle bundle of *in vivo* muscle spindles provides some information about the mechanical responses elicited by the stimulation of single fusimotor axons. In the preparation used, intact blood supply to the spindles has been found to be an essential condition for the maintenance of good responsiveness of the primary endings. If the blood supply is impaired, the tonic response of the primary

ending to repetitive stimulation of a fusimotor axon quickly decreases then disappears, while the dynamic response of the primary ending and the contraction of the intrafusal muscle bundle persist for a long time. The observations of partly isolated muscle spindles with intact blood supply have been taken into account only when the primary ending responses to fusimotor stimulation were similar in form to the ones observed before the dissection.

Effects of the dissection on resting discharge of the primary ending

During the dissection, the tenuissimus muscle segment containing the spindle was maintained at its physiological length. However, the rate of resting discharge of the primary ending was often less after the dissection. An abrupt decrease in the frequency of firing was observed upon sectioning connective tissue close to the capsule or the poles. The manner in which the connective tissue components attach to the intrafusal muscle fibres is not known precisely, nor is the way in which the extrafusal forces are transmitted to the intrafusal structures. Sometimes the muscle spindle dissection caused, without any prominent muscle spindle injury, the resting primary ending discharges to cease, but the response of this ending to fusimotor axon stimulation was unchanged.

Relative independence of movements of intrafusal muscle fibres

During fusimotor stimulation, an ability of the intrafusal muscle bundle to slide easily within the capsule and an ability of one bag fibre to slide relative to another was found. This seems to be an important feature of the intrafusal mechanics. The connective tissue is probably organized so as to allow these displacements to take place easily. When the intrafusal muscle bundle contains two nuclear bag muscle fibres, a commonly observed occurrence, contraction of one of these fibres in one pole never led to appreciable transmitted movement in the same pole of the other fibre. In this experimental situation and possibly under physiological conditions (low-frequency activation) mechanical interactions arising from the parallel arrangement of the bag fibres do not seem to exist. Whether each chain fibre has the same ability to slide relative to another is not so easy to establish. On many occasions, during the stimulation of a static γ axon, a movement was induced in several chain fibres. It was difficult to ascertain in some of these fibres if the movement had been induced by a common innervation or by a mechanical coupling between contiguous chain fibres. Indeed, anatomical findings indicate that in equatorial and juxta-equatorial regions the chain fibres are closely joined by a series of button-like attachments (zonulae adhaerentes; Corvaja, Marinozzi & Pompeiano, 1967; Adal, 1969). Two chain fibres may be attached at some distance

from the equatorial region by one endomysial cell or by close apposition of two endomysial cells (Landon, 1966; Corvaja, Marinozzi & Pompeiano, 1969). Thus chain fibres can act in parallel, while bag fibres which are not directly connected to each other or to chain fibres cannot. Sometimes, particularly with a high rate of static fusimotor stimulation, the movements induced in chain fibres are transmitted to the extremities of the bag fibres. In this respect it must be recalled that the terminal endomysium of a chain fibre at times is not attached to the capsule in the usual manner, but instead merges with the endomysium of a neighbouring bag fibre; this should lead to an unloading of the equatorial region of bag fibres but we never found any clear evidence of shortening of the bag equatorial region even when the region was clearly visible.

Propagated and localized contractions

One third of static γ axons evoke contractions that seem to involve the whole pole of the chain fibres which they innervate. Possibly these static axons are representative of static axons described by Bessou & Pagès (1972) the stimulation of which elicits propagated action potentials in intrafusal muscle fibres. This hypothesis is supported by the experimental findings of Barker, Bessou, Jankowska, Pagès & Stacey (1975, and unpublished observations) that the stimulation of four static γ axons evoked action potentials in three intrafusal chain fibres and one intermediate bag fibre, i.e. a bag fibre with sarcomere M band as exists in nuclear chain fibres (see Barker, 1974).

The remaining static γ axons and the dynamic γ axons elicit one or several foci of convergent movements of sarcomeres in the intrafusal muscle fibres. These movements could be representative of localized contractions, more especially as Bessou & Pagès (1972) have shown that two thirds of static γ axons and all dynamic γ axons evoke junction potentials in intrafusal muscle fibres. Such experiments have made it obvious that junction potentials were almost always derived at the level of or in the close vicinity of convergent movements in the spindle poles.

Contractile features of intrafusal muscle fibres

Chain fibres. Our observations show that chain fibres contract and relax more rapidly than bag fibres. This corroborates the observations made in isolated mammalian spindles by either stimulating directly the intrafusal fibres (Smith, 1966) or indirectly activating with different stimulus strengths unidentified axons in the muscle nerve (Boyd, 1966).

Bag fibres. In bag fibres innervated by dynamic γ axons no transient movement was discernible in response to a single shock or a low rate of

fusimotor stimulation. However, from other evidence it is known that each stimulus evokes a junction potential in intrafusal fibres (Bessou & Pagès, 1972) and often a transient increase of the primary ending discharge (Bessou, Laporte & Pagès, 1968*b*). The contractions of bag fibres innervated by dynamic γ axons are weak and slow. They are the weakest and the slowest of intrafusal muscle fibre contractions. These contractile characteristics of bag fibres innervated by dynamic γ axons look like those of tonic muscle fibres of the frog (Kuffler & Williams, 1953) and the slow fibres of extraocular fibres of the cat (Hess & Pilar, 1963).

The bag fibres supplied by static γ axons contract in response to a single nerve stimulus. The contractions of these fibres are stronger and quicker than those of the bag fibres innervated by dynamic γ axons but weaker and slower than those of the chain fibres.

Histochemical and ultrastructural works (see Barker, 1974) support the existence of two kinds of nuclear bag fibres: typical nuclear bag fibres and intermediate nuclear bag fibres. Particularly, the latter type has a sarcomere M line like chain fibres. The intermediate bag fibres could be the bag fibres with the quicker and the stronger contractions and innervated by static γ axons while the typical bag fibres the ones with the weaker and the slower contractions and innervated by dynamic γ axons. However, such a speculation does not fit well with the histochemical profile of cat's intermediate bag fibres, that are in this respect farther from the chain fibres than from the typical bag fibres (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1975), and is invalided by histophysiological investigations. Barker, Emonet-Dénand, Harker, Jami & Laporte (1974*a*, *b*) have shown that stimulation of four dynamic γ axons produced glycogen depletion sites in seven typical bag fibres and in sixteen intermediate bag fibres of seventeen muscle spindles. The stimulation of seven static γ axons elicited in twenty muscle spindle poles glycogen depletion sites in twenty-one bag fibres comprising both typical and intermediate types. Barker, Bessou, Jankowska, Pagès & Stacey (1975, and unpublished observations) have found that of seven bag fibres innervated by static γ axons and marked with Procion yellow three were typical and two intermediate bag fibres; the form of the two remaining bag fibres was not determined because the presence of the M band in the sarcomeres has not been searched for. Five bag fibres innervated by dynamic γ axons were all typical bag fibres.

Distribution of fusimotor γ axons to intrafusal muscle fibres

Some years ago Jansen & Matthews (1962) suggested that bag fibres were the main support of the dynamic sensitivity of primary endings. The suggestion was based upon some unproved assumptions about different

mechanical properties of both kinds of intrafusal muscle fibre and intrafusal mechanics. Matthews (1962) suggested that static and dynamic γ axons achieve their distinct actions by respectively supplying the chain and the bag fibres. Matthews (1972) in the light of certain evidence suggests there is a 'widespread feeling' that this is still so.

This work shows that the pattern of distribution of fusimotor γ axons is more complex. All dynamic γ axons innervated bag fibres but in many spindles the stimulation of several static γ axons elicited contractions in bag fibres which were never found to contract when dynamic γ axons were stimulated. In some instances several static γ axons activated the same bag fibre. It can be stated that about two thirds of static γ axons supplied bag fibres and that at least one third of static γ axons supplied both bag and chain fibres. It is highly probable that this latter figure is an underestimate: (i) for twelve static γ axons supplying bag fibres a possible concomitant innervation of chain fibres could not be ascertained owing to technical difficulties; (ii) intrafusal fibres the diameters of which were in the range 13–18 μm have not been included in the results since they could not be identified either as chain or as bag fibres. Among these fibres most were probably bag fibres.

Providing the nature of stimulated fusimotor axons is not taken into account, our observations may be compared to some extent to those of Boyd & Ward (1975) that described the distribution of 148 fusimotor axons the static or dynamic function of which was not identified. However, some quantitative differences do exist in the respective conclusions. Boyd & Ward found 89 % of fusimotor axons produced visible contractions in either nuclear bag fibres or nuclear chain fibres and 11 % only of fusimotor axons evoked contractions in both types of intrafusal fibres (versus 22 % of all the fusimotor axons in our work). The reliability of these conclusions may be discussed for the following reasons: (i) the observations have been made on *in vitro* isolated muscle spindles; (ii) relatively low microscopic magnification has been used for detailed observations and photographic recordings; (iii) as indicated by the authors the isolation process of muscle spindles can easily result in partial denervation of some intrafusal fibres and especially of nuclear bag fibres; (iv) motor stimulation has been performed not on filaments containing only one fusimotor axon but, on the contrary, on the tenuissimus muscle nerve close to the spindle by progressively increasing the strength of the stimulus. Under these conditions, two fusimotor axons of the same threshold may be simultaneously recruited, a branch originating from a fusimotor axon dividing above the electrode of stimulation may be stimulated apart from the other branch; moreover, a certain number of stimulated axons were skeleto-fusimotor axons; (v) recruiting of fusimotor axons with increasing strength of

stimulation provokes in muscle spindles cumulative contractile phenomena more and more complex and increasingly difficult to interpret. For instance, the contractions elicited in some intrafusal fibres by stimulating a high threshold fusimotor axon may be made indistinct in one or several fibres owing to the intrafusal contractions simultaneously produced by the stimulation of low threshold fusimotor axons.

Boyd, Gladden, McWilliam & Ward (1973) using an experimental preparation similar to the one used in this work (*in vivo* muscle spindle of abductor quinti digiti muscle of the cat) found a pattern of distribution of static and dynamic axons fairly similar in some aspects to the pattern of distribution we have established. All dynamic γ axons evoked local contractions of bag fibres only. Half of the static γ axons elicited contraction of chain fibres only. The other half of static γ axons simultaneously supplied the two types of intrafusal fibres but in this distribution pattern the static γ axons innervated mainly chain fibres with accessory innervation of bag fibres. This last statement is not corroborated by the observations described in this paper. Furthermore, no static γ axons are mentioned innervating bag fibres only. Finally the percentage quoted may be unreliable owing to the small number (twelve) of fusimotor axons which were prepared.

Histophysiological investigations show that bag fibre innervation by static γ axons is not uncommon. In the works of Barker and his colleagues in co-operation with Bessou and his colleagues (Barker, Bessou, Jankowska, Pagès & Stacey, 1972, 1975, and unpublished observations), thirteen intrafusal fibres activated by single static γ axons have been marked by Procion yellow injection and then examined under the electron microscope. Five were chain fibres and seven bag fibres.

In preceding works, the distribution of fusimotor axons has been established in only one muscle spindle of the muscle spindle cluster that each fusimotor axon was innervating. Recent histophysiological works studied the quantitative complete distribution of static γ axons on the intrafusal muscle fibres of all spindles supplied by each axon. Barker, Emonet-Dénand, Laporte, Proske & Stacey (1971, 1973) have determined the distribution pattern of static γ axons in the tenuissimus muscle of the cat the motor innervation of which was reduced to one single γ axon by section and consequent degeneration of all the other motor axons. In the six cases examined the surviving γ axons supplied between them thirty muscle spindles. Each static γ axon innervated from three to seven muscle spindles and was connected by trail endings with both chain and bag intrafusal fibres. The static γ axons supplied chain fibres alone in eight spindles, bag fibres alone in seven spindles, and both chain fibres and bag fibres in fifteen spindles. From this study it was also ascertained that the

'fusimotor unit' (i.e. the totality of intrafusal muscle fibres innervated by a single fusimotor axon) of static γ axons is *always* made of chain and bag fibres.

Brown & Butler (1973) using the glycogen depletion technique have shown that dynamic γ axons innervate preferentially bag fibres and occasionally chain fibres. Furthermore, from their results it may be deduced that any static γ axon is distributed to both bag and chain fibres when the totality or a large number of the spindles supplied by each axon is taken into account. The percentage of spindles with both chain and bag fibres supplied by a static γ axon is as high as 75 %. Boyd & Ward (1975) consider this latter estimate too high and suspect that some glycogen depletion sites were not due to fusimotor stimulation but were attributable to the difficulty in achieving adequate fixation with this method. To avoid this criticism, Barker *et al.* (1974*a*, *b*) have quickly removed and then directly afterwards frozen the tenuissimus muscles in iso-pentane cooled to $-160°$ C; the muscles were then fixed in absolute alcohol at $-30°$ C for 3 days and embedded. Serial transverse sections were stained for glycogen using the PAS method. The results obtained in this work agree with those of Brown & Butler (1973).

Considerations of the functional implications of fusimotor axon distribution

From the observations of Boyd *et al.* (1973) and from their own experiments Brown & Butler (1973) infer that 'the dynamic effects can occur with bag fibre contraction alone and static effects with chain fibre contraction alone, but not vice versa'. Such an assertion is correct concerning the dynamic effects since dynamic γ axons innervating only chain fibres have never been found. Concerning the static effects the assertion is inaccurate. In the present work it was unquestionable that three static γ axons elicited contractions only in bag fibres. Possibly some of the twelve static γ axons whose stimulation produced contractions in bag fibres but whose contingent innervation of chain fibres was not searched for were also distributed to bag fibres only. In the histophysiological works of the teams of Barker & Laporte (Barker *et al.* 1971, 1973) it clearly appears that in seven out of thirty muscle spindles studied, single static γ axons innervated bag fibres only. In other spindles these same axons were supplying either only chain fibres or both chain and bag fibres. Since one axon exerts the same kind of functional action on all the muscle spindles it innervates (Crowe & Matthews, 1964; Brown, Crowe & Matthews, 1965; Bessou, Laporte & Pagès, 1966), it may be asserted that static effects exerted by a single γ axon are not related to the distribution pattern of the axon on the various types of intrafusal muscle fibres.

The functional meaning of simultaneous contractions of bag and chain

intrafusal fibres elicited by the stimulation of some static γ axons is not clear. Brown & Butler (1973) suggested that 'the inevitable unloading generated in the central portions of bag fibres by chain fibre activation is partly taken up by the simultaneous bag contractions. This may leave the spindle in a better state to respond quickly to a change from static to dynamic activation or may assist the dynamic γ effects to come through when both sorts of γ are activated simultaneously.' In the present work unloading of the central region of bag fibres produced by the contraction of chain fibres was never observed. However, it cannot be assumed that such an unloading does not appear when all static γ axons innervating a spindle are activated as this possibly occurs in physiological conditions. Moreover, in the internal working of the muscle spindle proposed by Brown & Butler (1973) any offset of the unloading is efficient only if the same bag fibre, in a given spindle, is simultaneously supplied by static and dynamic γ axons. Such an occurrence was never found in our experiments. Indeed most of the time the tenuissimus muscle spindles contain two nuclear bag fibres, one innervated by one or several static γ axons, the other by a dynamic γ axon.

The innervation by some fusimotor axons of both types of intrafusal fibre could explain why the stimulation of these axons may exert on primary endings either a static action when the rate of fusimotor stimulation is high or a dynamic action when the rate of fusimotor stimulation is low (Emonet-Dénand, Joffroy & Laporte, 1972). These authors suggest that at low frequency of stimulation the contraction of bag fibres could increase the deformation of their sensory terminals during phasic stretch, producing a dynamic effect, while at a high frequency of stimulation the powerful contraction of chain fibres could unload the central region of bag fibres counteracting the effects of the bag fibre contraction and of the stretch on the sensory terminals situated around nuclear bags. In this manner the phasic sensibility of primary ending could be reduced. The preceding explanation requires that the nuclear bag fibres contract more slowly than the nuclear chain fibres innervated by the same static γ axon. The observations reported in the present paper support this statement.

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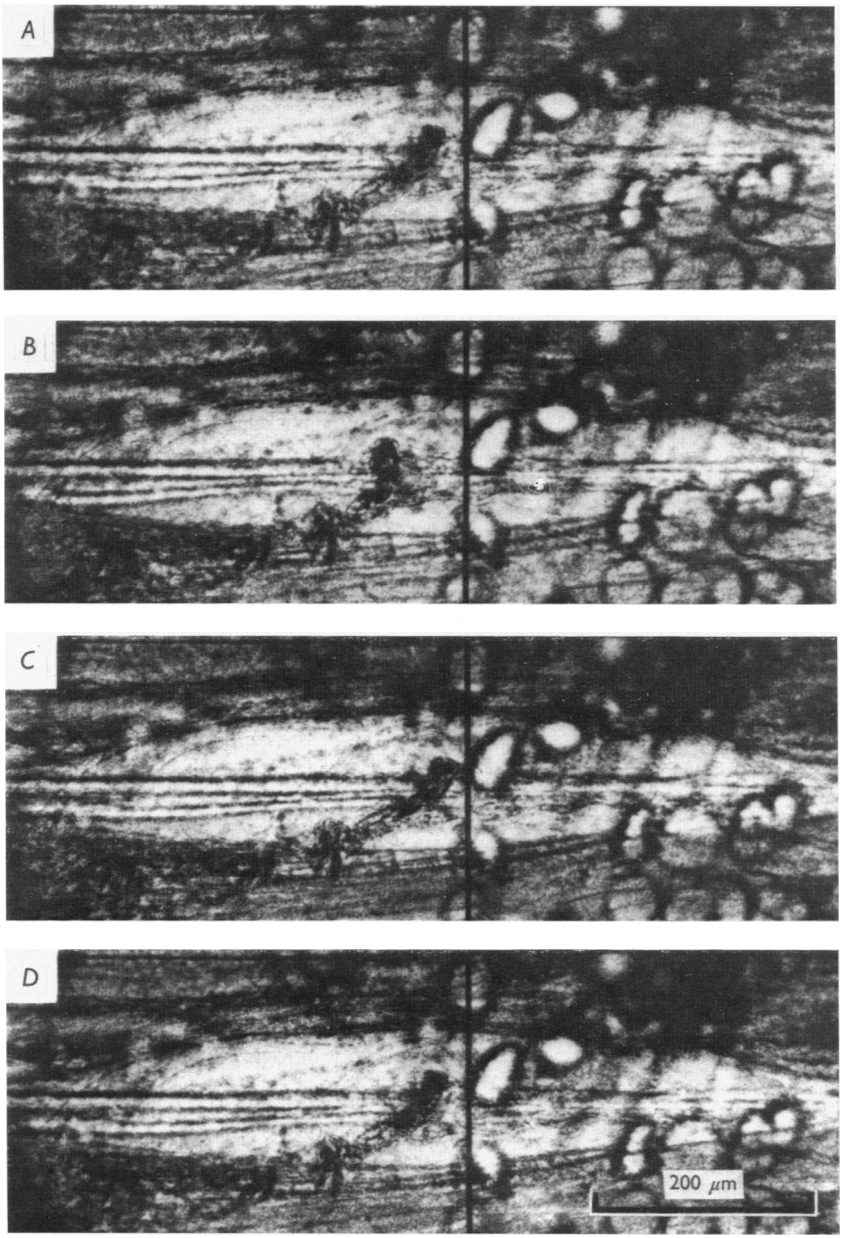
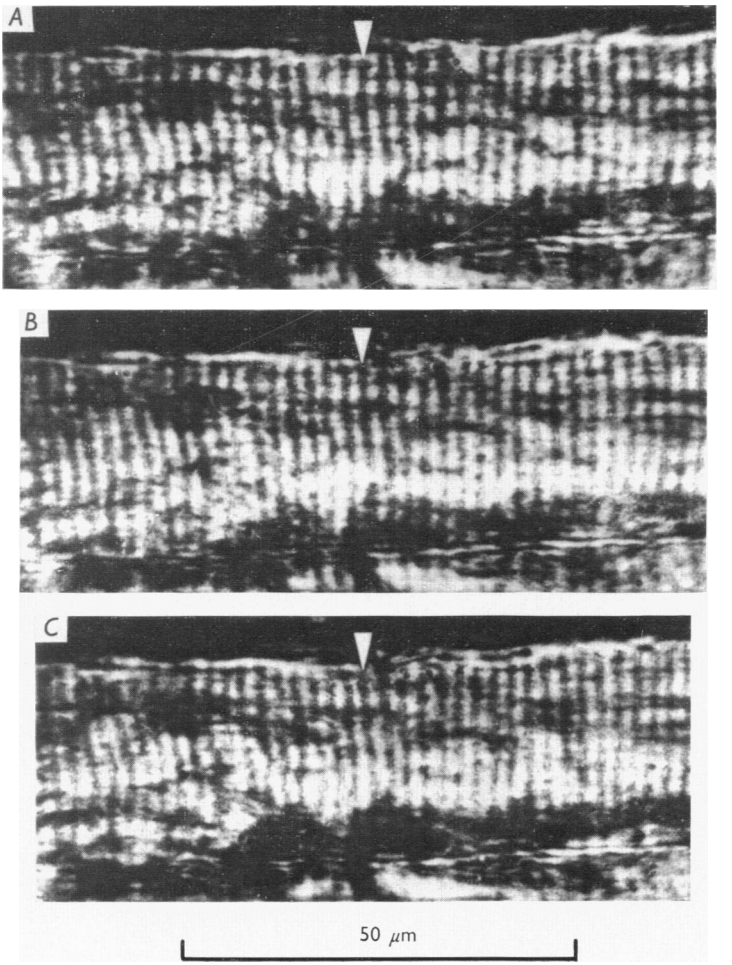

A
B
C
D
200 μm


A
B
C
50 μm

EXPLANATION OF PLATES

PLATE 1

Microphotographs, selected from a cine film, of the intracapsular region of an *in vivo* muscle spindle of cat tenuissimus muscle. The oblong clear region is the periaxial space. The bundle of intrafusal muscle fibres crosses the periaxial space. The spindle nerve coming from the tenuissimus muscle nerve runs obliquely upward and to the right in the space and finally meets the intrafusal muscle bundle. The meeting point is used as reference point. Muscle spindle no. 16.

Fig. *A*. Muscle spindle at rest. No fusimotor stimulation.

Fig. *B*. Stimulation at a rate of 110/sec of a single static γ axon (conduction velocity: 34·1 m/sec). Maximal displacement of the reference point of 45 μm towards the left.

Fig. *C*. Stimulation at a rate of 110/sec of another single static γ axon (conduction velocity: 39·0 m/sec). Maximal displacement of the reference point of 14 μm towards the right.

Fig. *D*. Stimulation at a rate of 110/sec of a single dynamic γ axon (conduction velocity: 35·8 m/sec). No visible displacement of the reference point.

PLATE 2

Microphotographs, selected from cine film, of thirty-six successive sarcomeres of a nuclear bag fibre (width 25 μm). The sarcomere sequence, 0·78 mm distant from the equatorial reference point, was the central part of a local contraction when a single static γ axon (conduction velocity: 35·1 m/sec) was stimulated. The shortening of the sarcomere sequence appeared in the guise of convergent movements of lateral striations towards an immobile central striation (white triangle) and a decrease of the length of the thirty-six sarcomere sequence. Muscle spindle no. 15.

Fig. *A*. Resting length of the thirty-six successive sarcomeres.

Fig. *B*, *C*. Lengths of the thirty-six successive sarcomeres after respectively 0·2 sec and 1 sec of repetitive stimulation at 90/sec of the static γ axon. In fig. *C* the shortening was at a maximum.