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STUDIES ON THE SITE OF TERMINATION OF STATIC AND DYNAMIC FUSIMOTOR FIBRES WITHIN MUSCLE SPINDLES OF THE TENUISSIMUS MUSCLE OF THE CAT

BY M. C. BROWN AND R. G. BUTLER*
From the University Laboratory of Physiology, Oxford OX1 3PT

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SUMMARY

1. The site of termination of static and dynamic fusimotor fibres has been mapped by finding which intrafusal muscle fibres have been depleted of glycogen as a result of tetanic stimulation of single γ fibres. Long periods of stimulation coupled with occlusion of the blood supply were necessary to cause glycogen depletion.

2. In cat tenuissimus muscle, dynamic γ motor fibres always activated bag intrafusal muscle fibres, and occasionally chain fibres. Static γ fibres always activated chain fibres and frequently activated bag fibres as well.

3. It is argued that these results can be fitted into the hypothesis of the mechanism of internal functioning of the spindle originally proposed by Jansen & Matthews (1962). It is also pointed out that the results raise problems concerning the mechanism of development of the spindle motor innervation.

INTRODUCTION

Mammalian muscle spindles contain at least two distinct kinds of intrafusal muscle fibres, the nuclear bag and nuclear chain fibres, which can be differentiated on gross morphological, histochemical and ultrastructural grounds (see review by Matthews, 1972). Furthermore, they contract at different speeds (Smith, 1966; Boyd, 1966). Muscle spindles are also innervated by two functionally distinct kinds of fusimotor fibre, the static and dynamic γ fibres of Matthews (1962).

The simplest plausible scheme which combined these two separate groups of experimental results (and in some respects predated them) is that of Jansen & Matthews (1962) (see also Brown & Matthews, 1966; Matthews, 1972) who proposed that dynamic γ fusimotor fibres innervated the bag intrafusal muscle fibres and static γ fusimotor fibres innervated

* Fellow of the Multiple Sclerosis Society of Canada.

the chain intrafusal muscle fibres. The very different effects of the two sorts of fusimotor fibre on the primary ending's response to stretch were attributed to the different mechanical properties of the bag and chain fibres. In addition, independent selective innervation of the bag and chain fibres was also supported histologically in two different species (Boyd, 1962, for cat; Porayko & Smith, 1968, for rat). Independent innervation would also be expected on developmental grounds (although this line of argument has never been advanced); for there is a large body of evidence which shows that particular muscle fibre types are associated with motoneurones of a particular type, that nerves are 'matched' to muscle (Mark, 1969), and that inappropriate innervation is rejected (Marotte & Mark, 1970*a*, *b*).

Meanwhile, however, anatomical evidence has been produced by Barker and his colleagues (e.g. Barker, Stacey & Adal, 1970) that single γ fusimotor fibres end within the spindle with terminals on both sorts of intrafusal muscle fibre. The strongest evidence in favour of such non-selective innervation was obtained in collaboration by Barker, Emonet-Dénand, Laporte, Proske & Stacey (1970). These authors cut all but one fusimotor fibre innervating the cat tenuissimus muscle, allowed time for the other motor fibres to degenerate and then determined that fusimotor fibre's termination sites by silver impregnation. In these experiments only static fusimotor fibres were studied but the unequivocal finding was that such γ fibres had terminals on *both* bag and chain fibres more frequently than not.

A possible reconciliation of these two contrasting viewpoints was suggested by Brown (1972) and independently by Smith & Ovalle (1972). The proposal was that when a γ fibre had branches on both sorts of intrafusal fibres, only one set of terminals was capable of transmitting, the set on the other type of intrafusal muscle fibre having been in some way 'inactivated' by the 'correct' motor fibre. The precedent for such a state of affairs is found in the experiments of Marotte & Mark (1970*a*, *b*), who showed that motor nerves to extrinsic eye muscles of fish could innervate an eye muscle other than that which they normally supplied only if the 'correct' motor fibre was cut. If the original motor fibre subsequently reinnervated the muscle, the foreign terminals ceased to work but remained on the muscle with apparently normal ultrastructure. Given the possibility of a comparable situation within the spindle, histology on its own cannot provide a final answer to the problem of the functional distribution of γ fibres in muscle spindles.

Direct evidence for activation of an intrafusal fibre can be obtained either by observing its contraction or by recording its electrical activity with a micro-electrode. Both of these methods require isolation of the

spindle. An alternative method is to use a modification of the glycogen depletion technique of Edström & Kugelberg (1968). In outline, the method is to stimulate a fusimotor fibre for a period of time in order to cause the intrafusal muscle fibres which that motor fibre innervates to contract and use glycogen, so that subsequent staining of the whole spindle for glycogen will reveal the distribution of that fusimotor fibre by the presence of intrafusal muscle fibres containing less than normal amounts of glycogen. The results which we describe in this paper with the use of this technique are all from the tenuissimus muscle where localization of the activated spindles is easy. As briefly reported earlier (Brown & Butler, 1973) the results show that non-selective innervation of intrafusal muscle fibres does occur.

METHODS

Cats and rabbits were used. The former were anaesthetized with pentobarbitone sodium (Nembutal) at an initial dose of 36 mg/kg given I.P. The rabbits were anaesthetized with an I.V. mixture of 25% urethane (7 parts) and Nembutal (1 part of a solution containing 60 mg/ml.).

The experiments were performed on the tenuissimus muscle which was dissected and its spindles localized by the methods described by Bessou & Laporte (1965). Stimulating, recording and muscle stretching equipment were all conventional.

In nine experiments the whole muscle nerve was stimulated at strengths of $10 \times \alpha$ threshold. For most experiments, a laminectomy was performed in order to split the dorsal roots into filaments containing single primary spindle afferents from the tenuissimus. The ventral roots were split as well into a large number of filaments and each was tested for a fusimotor action on one of the afferents. It was usual to find three filaments with a static action and one with a dynamic action on each spindle. One of these filaments was then chosen and subdivided until it contained only a single functional γ fibre supplying the tenuissimus. The criteria for singleness were the presence of an all-or-none action potential in the ventral root filament at threshold stimulation of the nerve to tenuissimus, and absence of other potentials on increasing the strength up to $10 \times$ threshold. The action potentials were displayed on a Tektronix 5103 storage oscilloscope and the latency measured directly from this.

The criteria for deciding if the selected γ fibre was of the static or dynamic type were based on the behaviour of the primary ending during ramp stretches and releases. Static fusimotor fibres decrease the dynamic index during stretching, and during release enable the primary ending discharge to continue (Crowe & Matthews, 1964*b*). Dynamic fibres increase the dynamic index, and during release the spindle discharge goes silent or drops to a new steady level. Other criteria are that the static fibres often increase the irregularity of discharge and usually have a strong excitatory action with the muscle held at constant length, while dynamic fibres often only have a weak excitatory action on the discharge rate when the muscle is not being stretched.

Regime used for producing glycogen depletion

The functionally single γ fibre was stimulated tetanically at either 80 or 100/sec in bursts lasting 1·7 sec, the burst frequency being one every 3 sec. The stimulation

was interrupted for a rest period of about 20 sec in every 50 sec. The total time for which this pattern of stimulation was applied was usually 3 hr. Higher frequencies of γ stimulation or tetanic bursts of longer duration led to γ failure. The strength of stimulation was set at twice the threshold value.

During stimulation the blood supply to the tenuissimus was reduced by pulling with 50 or 100 g weights tied on to a ligature looped over the femoral and deep femoral arteries. This rarely stopped the capillary flow completely, in the tenuissimus and the weights could be left on for up to 0·5 hr, with a break of 5 min before reapplication.

Histology

Most of the spindles studied lay in the tenuissimus distal to the point of nerve entry. The precise localization of the activated spindles was confirmed at the end of each experiment by tying ligatures first distal and then proximal to the spindle, while listening to the spindle discharge. If the discharge ceased on tying the second ligature it was presumed that the spindle lay between the two ligatures. This was checked histologically. The length between the ligatures was usually about 1 cm. Up to five spindles activated by a single γ fibre could be isolated in each experiment.

The muscle was then cut out and fixed in Rossman's fluid (9 parts 90 % ethanol saturated with picric acid: 1 part formalin) and left overnight in this solution at 4° C. It was then dehydrated in ethoxyethanol and the segments containing spindles were embedded in ester wax and serially sectioned at 8 μm. Staining for glycogen was carried out using the periodic acid–Schiff method as modified by Bulmer (1959) to include a dimedone blockade, which makes the staining for glycogen specific. When fixed in this way, 'streaming' of the glycogen to one side of the extrafusal fibres was kept to a minimum. Such streaming did not occur at all in the intracapsular portions of intrafusal fibres but was sometimes seen in the more peripheral parts of bag fibres. Speed of fixation is probably of great importance for accurate estimates of glycogen content, for during slow fixation it is possible for glycogen to be metabolized (Schabadasch & Schabadasch, 1972). We have evidence for this in two experiments where, in an attempt to further reduce the 'streaming' in extrafusal fibres, the tenuissimus muscles, both experimental and control, were embedded in liver before fixation in the hopes of obtaining more uniform entry of fixative around the fibre perimeters. This slowed fixation and in control spindles there was certainly a reduction in glycogen content. The results from these two experiments are not included.

Examination of the sections was made at 50 μm intervals, and a serial reconstruction made of each spindle studied to show the disposition and glycogen content of individual intrafusal fibres. In this way the identity of each fibre (bag or chain) was established and the presence and extent of glycogen depletion noted.

The tenuissimus from the other leg was simultaneously fixed and sectioned to provide a standard with which the glycogen content of the experimental spindles could be compared. In most experiments the control leg had the blood supply occluded in the same way as the experimental, and the muscle was also tied off by ligatures into strips 1 cm long before fixation, the ligatures being in this case arbitrary in position. The experimental and control sections were stained simultaneously. In a few experiments the experimental and control muscles were embedded in the same block and thus stained on the same slide. There appeared to be no difference between control muscles and muscles simply removed and fixed directly, without occlusion of the blood supply.

Pharmacological agents

In one experiment intrafusal contraction was induced by repeated injections of suxamethonium chloride (400 μg/kg). A total of twelve I.V. infusions of this dose was given over a period of 2·5 hr. Spontaneous respiration was allowed to recover in between injections.

In five experiments gallamine triethiodide was given I.V. in a dose varying from 5 to 20 mg/kg. The cats were decerebrated before the administratien of these drugs.

RESULTS

Glycogen distribution in normal tenuissimus spindles

In most unstimulated spindles of the tenuissimus muscle all the intrafusal fibres stain as intensely for glycogen as the surrounding extrafusal fibres. The glycogen is uniformly distributed from the polar ends up to and including the juxta-equatorial region. In the equatorial region the glycogen often appears to be completely absent or to be present in a thin peripheral rim around the fibre.

The second most common pattern is for the glycogen to be distributed as above, but with bag fibres slightly less intensely stained than the chains, especially in the juxta-equatorial region.

In roughly one third of unstimulated spindles, one bag fibre would have one or rarely two short segments (max. 100 μm in length) which were much paler than the other bag fibre or fibres, or the rest of the fibre over the other parts of its length. Chain fibres never showed such isolated pale segments, and neither did more than one bag fibre per spindle.

Effects of whole nerve stimulation on intrafusal glycogen

In rabbits, stimulation of the nerve to tenuissimus at strengths $10 \times \alpha$ threshold at a frequency of 80/sec in the intermittent fashion described above caused complete depletion of glycogen in all intrafusal fibres in 0·5 hr. This period of stimulation was quite ineffective in cats, and, further, we found that high-frequency stimulation on its own, however prolonged this might be, did not necessarily lead to glycogen depletion. In this respect the intrafusal fibres resemble the C type extrafusal fibres of Edström & Kugelberg (1968), whose glycogen content was not decreased by tetanic stimulation. However, reduction of the blood supply as described above greatly increased the probability of finding glycogen-depleted fibres after a 2–3 hr period of stimulation, but presumably because of a variation in the effectiveness of the occlusion, we still have preparations in which there is no obvious reduction in spindle glycogen content.

Pl. 1 shows photomicrographs of transverse sections through spindles taken from the two tenuissimus muscles of one cat, one muscle (Pl. 1*a*)

having had its nerve stimulated at $12 \times \alpha$ threshold for 2·75 hr at 75/sec, and the other (Pl. 1*b*), the control muscle, having had no stimulation, but having been treated to the same period of blood occlusion as the experimental. All the intrafusal fibres in the experimental muscle are very lightly stained (contain very little glycogen) in complete contrast to the control muscle. With such whole nerve stimulation there seems to be little difference in the glycogen depletion of bag and chain fibres. Pl. 1 also shows that extrafusal fibres in the experimental muscle, although paler than control extrafusal fibres, are not devoid of glycogen. This is probably so because transmission at the extrafusal neuromuscular junctions fails rather quickly with the particular stimulus regime which is effective for the intrafusal fibres. Thus the extrafusal fibres in this situation probably contracted for much shorter periods of time.

Effect of stimulation of single γ fusimotor fibres

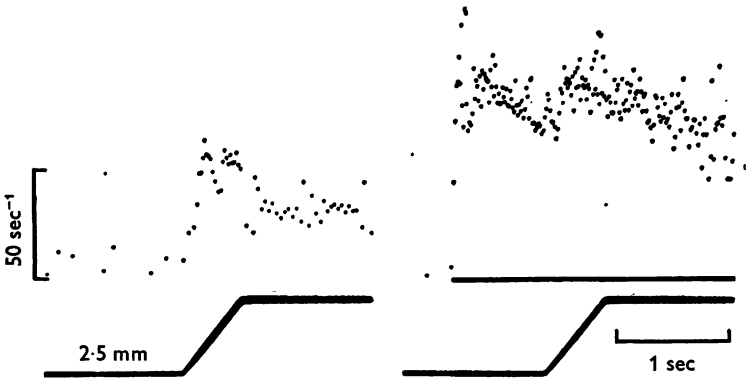
No attempt has been made to accurately quantify the degree of depletion. In certain fibres it was quite clear that the glycogen content was well below normal, these fibres appearing almost white. Such depletion had to extend at least over an arbitrarily selected distance of 200 μm in order to be considered significant. In other fibres it was quite clear that there had been no depletion, the staining being as intense as in controls. In between these two groups there were relatively few fibres where it seemed that the staining reaction was less intense than normal, but nevertheless some glycogen was clearly still present. These fibres probably were activated by the γ fibres concerned, but the various factors involved in bringing about glycogen utilization had not been such as to cause much glycogen break-down. Such fibres have been labelled differently in Text-figs. 2 and 4.

Static γ fibres. Stimulation of single static fusimotor fibres usually resulted in glycogen depletion in both nuclear bag and nuclear chain fibres. An example is shown in Pl. 2*a* and *b*, where at one pole of the spindle, two out of the four chain fibres are devoid of glycogen and all three bag fibres are paler than normal. The effect which this static γ fibre had on the response to stretch of this particular spindle is shown in Text-fig. 1. This γ fibre also activated four other spindles whose glycogen content was studied. Sections through both poles of one of these is shown in Pl. 2*c* and *d*. In this spindle, both poles had depleted fibres. The same bag fibre was depleted at both poles, and three chains of one pole and two of the other. The one chain fibre not depleted at one pole was depleted at the other. Pl. 2*e* is a section near the equator of this spindle. Pl 2*f* is a section through the pole of a spindle from the control tenuissimus.

We have studied six single static γ fibres whose terminations in eighteen

spindles were mapped. Only two of these caused depletion in chain fibres alone. Unfortunately, in those two cases only one spindle activated by each γ was localized so we cannot say whether in any other spindles their terminations were restricted to chain fibres alone.

The results from all six static fibres are summarized in Text-fig. 2. In this diagram both poles of each affected spindle are shown (except where localization was poor and one pole was lost) with large circles representing the bag fibres and small circles the chain fibres. Glycogen-containing fibres are black, glycogen-depleted fibres white, and those fibres where a definitive

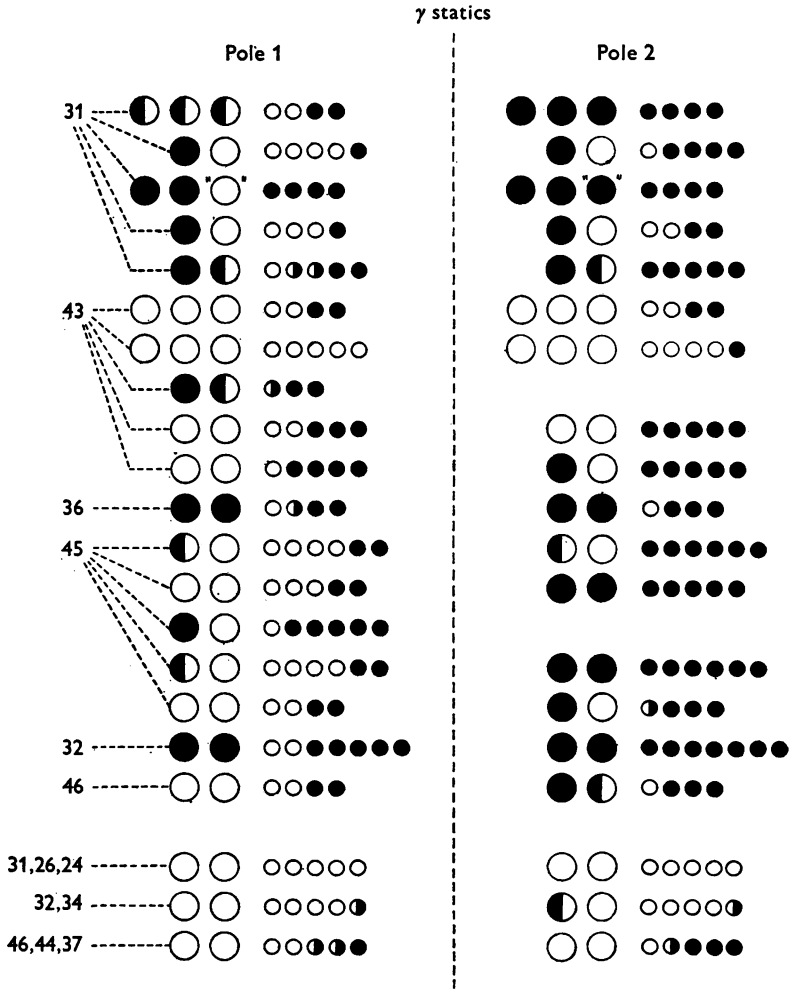


Text-fig. 1. Response to stretching of the spindle whose glycogen content is shown in Pl. 2*a* and *b*. On the right a stretch was applied during stimulation at 150 sec^{-1} of the single static fusimotor fibre used to deplete the intrafusal fibre of glycogen. Frequency display gives each action potential as a dot and its height is proportional to the reciprocal of the time interval since the preceding action potential: calibration bar for this is 50 sec^{-1}. Time bar 1 sec. Length of stretch 2·5 mm.

judgment was not possible (see above) are half black and half white. Where two poles of a spindle were affected to differing degrees, the most depleted is drawn on the left. The depleted fibres are drawn next to one another and centred about the gap in the diagram between the bag and chain fibres. Thus an intrafusal fibre at one position in pole 1 in the diagram is not necessarily the same fibre as the one drawn in the equivalent position in pole 2. The depleted fibres were grouped in this way for clarity. In those cases where depletion occurred at both poles, sometimes the same fibres were involved at both poles and sometimes different ones. There are ten possible cases in Text-fig. 2 where the same intrafusal fibres might have been involved at both poles: on five occasions the same fibres were involved; in three, different fibres were depleted at the two poles, and in

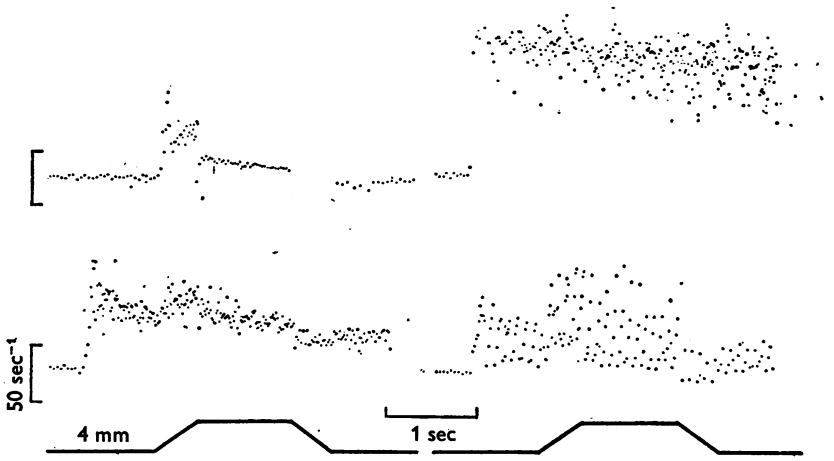
the remaining two cases tracing the fibres was too uncertain for a judgment to be made.

There is one example in Text-fig. 2 where a static fusimotor fibre apparently depleted only a nuclear bag fibre. This fibre has been drawn in quotation marks, for although it had a nuclear bag at the equator and



Text-fig. 2. Summary of the effects of stimulation of static γ fibres on spindle glycogen content. Results for six single static fibres whose conduction velocities in m.sec^{-1} are given beside the spindles which they innervated. ●, Bag fibre with normal glycogen; ○, depleted bag fibre; ◐, probably depleted bag fibre. •, chain fibres with normal glycogen content, o, depleted chain fibre; ◦, probably depleted chain fibre. At the bottom are results from three spindles which were depleted by stimulating two or more static γ fibres simultaneously. Full description in text.

was longer than the chain fibres, it was the shortest of the bag fibres in the spindle and the thinnest. It might therefore have been either an 'intermediate' fibre (Barker & Stacey, 1970) or an unusual chain. In three experiments, two or more static fibres going to a spindle were stimulated together, and the resulting depletions are shown at the bottom of Text-fig. 2. In two spindles there was complete depletion in all fibres. A section through one pole of one of these is shown in Pl. 3, and in Text-fig. 3, the effect of each of the three static fibres on this spindle is shown. The fact that all the bag fibres have been depleted on these two occasions seems to rule out the possibility that the bag fibres activated by static γ fibres are always 'intermediate' fibres.

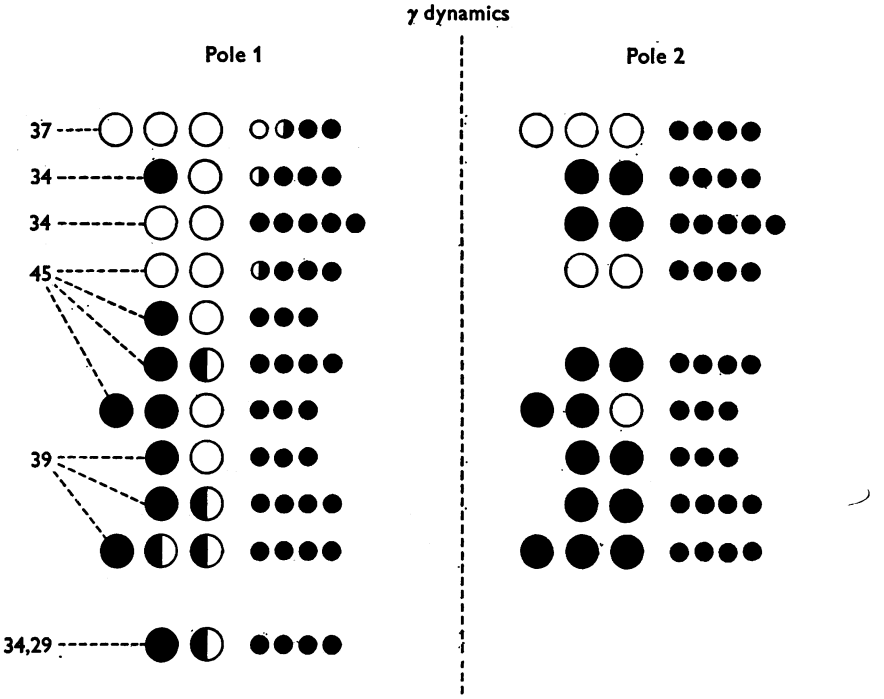


Text-fig. 3. Response to stretching of the spindle whose glycogen content is shown in Pl. 3*a*, to illustrate the action of the three static γ fibres used to deplete the intrafusal fibres of glycogen. Top left, response of passive spindle. Remaining three records during stimulation of each of the static fibres in turn at 150 sec^{-1}. Frequency display as in Text-fig. 1.

Dynamic γ fibres. The results from five single dynamic γ fibres and one pair of dynamic fibres stimulated simultaneously are summarized in Text-fig. 4, which is drawn up in the same way as Text-fig. 2. An example of the depletion is given in Pl. 4 and the effect that this dynamic fibre had on the response of this spindle to stretch is shown in Text-fig. 5. Dynamic fibres appear to be more selective than static fibres for the majority depleted only nuclear bag fibres. There is, however, evidence for weak activation of chain fibres on some occasions, as in the example illustrated in Pl. 4.

Comparison of Text-figs. 2 and 4 shows that there is a preponderance of spindles with large numbers of chain fibres in the static series, there being seven spindles with five or more chain fibres, whereas there is only one with five chain fibres in the

dynamic series. This may simply reflect our procedure for selecting γ fibres which is described above. Our impression is that dynamic γ fibres in the tenuissimus are not as powerful as those found in the cat soleus and their relatively weak action may only have shown up well during our isolation procedure in cats where spindles had a higher proportion of bag fibres to chain fibres.



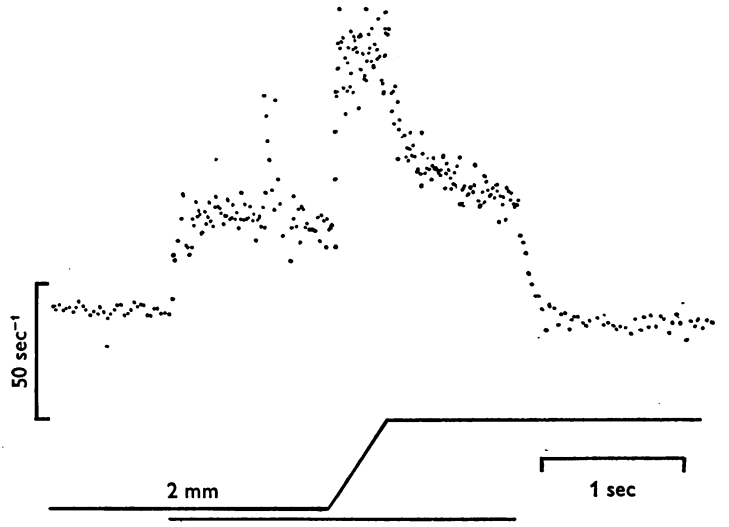
Text-fig. 4. Summary of the effects of stimulation of dynamic γ fibres on spindle glycogen content. Results for five single dynamic fibres and one pair of dynamic fibres. Conduction velocities in $m.sec^{-1}$ of the γ fibres are given beside the spindles which they innervated. ●, bag fibre with normal glycogen content; ○, depleted bag fibre; ◐, probably depleted bag fibre. •, chain fibre with normal glycogen content, ○, depleted chain fibre; ◦, probably depleted chain fibre. Full description in text.

Action of suxamethonium on glycogen content of spindles

As described above, we gave repeated large doses of suxamethonium intravenously in one experiment, and then examined the tenuissimus spindles for glycogen content. The control tenuissimus was taken out before the injections began. Both bag and chain fibres were depleted in the experimental muscle, but the bag depletion was more extensive and uniform.

Site of glycogen depletion in bag and chain fibres

The regions of intrafusal fibres depleted by static and dynamic γ fibres are not identical. Table 1 summarizes the length and position of the depleted region in the two sorts of muscle fibres as a result of static and dynamic fusimotor action. For each spindle, the mean length of depletion for chain and bag fibres was estimated, and an over-all series average calculated from this for each sort of fibre. The maximum and minimum



Text-fig. 5. Response to stretching of a spindle during stimulation at 150 sec^{-1} of the dynamic γ fibre which was used to produce glycogen depletion. The glycogen content of this spindle is illustrated in Pl. 4. Frequency display as in Text-fig. 1.

TABLE 1. Localizations of depleted regions for nuclear bag and chain fibres after tetanic stimulation of static and dynamic fusimotor fibres. The main figures are averages for all spindles, while those in parentheses indicate the extreme minima and maxima from individual spindles

	Bag fibres		Chain fibres	
	Length of fibre depleted (μm)	Distance from edge of nuclear region at which depletion starts (μm)	Length of fibre depleted (μm)	Distance from edge of nuclear region at which depletion starts (μm)
Static γ	620 (200 → 1480)	440 (0 → 1500)	600 (280 → 1120)	380 (0 → 980)
Dynamic γ	740 (300 → 1200)	740 (440 → 1680)	460 (3 samples only)	920 (3 samples only)

figures given in parentheses are the means for a particular spindle and not the maximum or minimum length in any one intrafusal fibre. It should be noted that these estimates are probably not very accurate, as there was some difficulty in deciding the exact border between depleted and undepleted regions. The main finding, which is in keeping with conventional histology (e.g. Barker, Stacey & Adal, 1970) is that dynamic fusimotor fibres tend to deplete a more distal site on bag fibres than do statics. On average, static fusimotor fibres deplete about the same length in bag and chain fibres.

The figures also show that on average the depletion does not extend from equator to pole tip in either sort of intrafusal fibre, because the length depleted is less than the length of either sort of intrafusal fibre. Taken at face value, this argues against propagated action potentials in either sort of intrafusal fibre. However, these are average figures, and in some chain fibres depletion was found over the entire length of one pole. Complete depletion was never found in bag fibres. These observations agree with the recent findings of Bessou & Pagès (1972) who have evidence from intracellular recordings that static γ fibres can cause propagated action potentials.

Lack of correlation of depletion with detailed action of γ on the afferent discharge

We have confirmed in these experiments the already well-documented fact (Brown, Crowe & Matthews, 1965; Bessou, Laporte & Pagès, 1966) that a given fusimotor fibre remains true to type, either a dynamic, or a static, in all the spindles on which it operates. Nevertheless, one might have expected to see some differences in their action from spindle to spindle which would correlate with the extent of their innervation as judged from the glycogen depletion, but none were noted.

Absence in the spindle discharge of evidence for activation of bag fibres by static γ fibres

It seems clear that although static effects can be produced in primary spindle afferents by contraction of chain fibres alone, the normal situation is for some bag fibre activation to occur at the same time. However, there seems to be no response due to this additional intrafusal contraction in the overall discharge from the spindle primary ending. It does seem reasonable to suppose that the contribution from bag fibre terminals of primary endings during static γ fibre stimulation should make its presence felt under appropriate circumstances by a change in the spindle discharge towards a more dynamic pattern. We have looked at two situations where this might be so.

Firstly during very large stretches starting from a long initial length it might be possible to see the bag fibre terminal output. We have, however, never seen the response of a spindle during static fibre stimulation become more velocity sensitive under these conditions.

Secondly, Emonet-Dénand & Houk (1968) demonstrated that following administration of gallamine triethiodide in a dose sufficient to block both static and dynamic γ fibres, dynamic fibres recovered first, and for a very long period dynamic γ fibres can successfully excite the spindle, but the static γ fibres cannot. It seemed possible to us that here static γ fibre branches on bag intrafusal fibres might be working while their chain branches were not. In five cats we investigated the effect of progressive gallamine blockade on the action of static fusimotor fibres on spindle primary endings during constant velocity stretches and releases.

Our results agreed with those of Emonet-Dénand & Houk (1968) in showing that static γ fibres could be blocked while dynamic fibres were not, although we never found that during the onset of block dynamic fibres were blocked before static and subsequently recovered. The excitatory effect of dynamic fibres gradually diminished in strength, and could in part be compensated by increasing the stimulation rate. This is to be expected where excitation-contraction coupling occurs through the medium of junctional potentials rather than propagated action potentials. The excitatory effect of static γ fibres could be greatly reduced without the response of the spindle ever becoming noticeably dynamic. However, high-frequency stimulation during gallamine blockade did not give a response which was identical to low frequency stimulation under normal conditions. During the block the discharge was more regular and the frequency of discharge rose more slowly at the start of stimulation. This again may probably be most simply attributed to the activation of the chain fibres during the block to be by graded junctional potentials rather than propagated action potentials leading to twitch contractions.

Summation of static and dynamic fibre stimulation was investigated on one spindle during partial gallamine blockade. The resulting frequency of discharge was higher than with either γ fibre alone, but the dynamic index (Crowe & Matthews, 1964*a*) was not increased. This sort of result is seen during combined stimulation of static and dynamic γ fibres under normal circumstances (Crowe & Matthews, 1964*b*).

DISCUSSION

The results described here are in agreement with a variety of other experiments. Firstly, classical histological techniques such as were used by Barker, Stacey & Adal (1970) and Jones (1966) show that fusimotor

fibres ending with trail endings usually have branches on both sorts of intrafusal muscle fibres. These trail ending fibres are presumably the static fusimotor fibres (see Matthews, 1972). On the other hand, fibres whose terminations end as plates (dynamic fusimotor fibres) are much more selective, and few have branches on chain fibres in addition to the branches on the bags. Secondly, direct observation of contractions in isolated spindles of the abductor digiti quinti medius muscle of cats has enabled Boyd, Gladden, McWilliams & Ward (1973) to observe that 50% of the static fusimotor fibres activate bags as well as chains. Thirdly, the combined physiological and histological experiments of Barker, Emonet-Dénand, Laporte, Proske & Stacey (1970) have shown that static fusimotor fibres usually end on bag and chain fibres. Lastly, Barker, Bessou, Jankowska, Pagès & Stacey (1972) very recently have shown that fibres labelled intracellularly with Procion yellow after recording their electrical activity during static fusimotor activation may turn out to be either bag or chain fibres.

All these results taken together with the present findings demonstrate that static fusimotor fibres may be non-selective in their termination sites and that, contrary to the prediction of Brown (1972) and Smith & Ovalle (1972), static fibre junctions on both sorts of intrafusal fibre may be functional.

Possible artifacts with glycogen-depletion technique

There are, however, certain discrepancies between the glycogen method and other methods of tracing fusimotor terminations which have been pointed out to us by Professors Barker and Boyd. Their data suggest that relatively few static fusimotor fibres innervate *both* poles of any given spindle, whereas our data (see Text-fig. 2) show that over 60% go to both poles. The question is: does this imply that glycogen depletion was occurring in our experiments other than when a fusimotor fibre was making certain regions of intrafusal fibres contract? The presence of some pale segments in control bag fibres might seem to point this way and the following are possible artifacts:

(1) Blood supply occlusion coupled with localizing thread ties (see Methods) might give rise to anoxic contraction, which would deplete glycogen. It is also known that slow fixation may lead to glycogen depletion (Schabadash & Schabadash, 1972).

(2) Blood supply occlusion might prevent the removal of potassium ions liberated from contracting intrafusal fibres, and these ions might in turn give depolarization and contraction in other intrafusal fibres (as suggested to us by Dr J. J. B. Jack).

(3) Pull of non-contracting portions of intrafusal fibres by other con-

tracting fibres might 'activate' the non-contracting fibres. It has been shown that heat output from the non-contracting frog sartorius rises with increase in muscle length (e.g. Clinch, 1968).

We believe that none of these occurred. The glycogen depletion method gave results in which, on occasions, only one pole of a spindle was affected. Furthermore, certain intrafusal fibres at each pole were *not* depleted. If the artifacts did occur, one would not expect to find an undepleted pole, or undepleted fibres. In addition, the pattern of depletion (dynamic to bag mainly, static to both) and the region of intrafusal fibre in which depletion occurs is different after static and dynamic fibre stimulation. There is no obvious mechanism whereby this could occur if the depletions were artifacts of the methods. If anoxic or potassium contractions had occurred we should also have seen a change in the resting discharge rate of the spindle and a change in its response to stretch during the course of the experiment. But such changes were not observed.

The difference between the number of double pole to single pole innervations which we find and which Barker and Boyd and their colleagues find may be attributed to some (or all) of the following factors. Firstly, it is doubtful if the difference is statistically significant. In a sample of only eight spindles, the likelihood of finding three or less than three spindles (Boyd *et al.* 1973) in which both poles are activated by static γ fibres is about 13% if the true proportion was 63% (double):37% (single) (our findings). Secondly, in our experiments we deliberately chose static γ fibres which had a powerful excitatory action in the hope of achieving greater depletion. Possibly, the more 'powerful' γ fibres innervate both poles more frequently. Thirdly, it is likely that all the methods currently available for tracing γ fibres to their termination sites are more liable to underestimate than over-estimate the proportion of spindles in which γ fibres go to both poles. In isolated spindles there is the risk of damage, and also the difficulty of seeing all the intrafusal fibres all along their lengths to detect what may be very small contractions (see Bessou & Pagès, 1972). In our experiments glycogen depletion is hard to achieve, so that failure to see glycogen depletion does not prove the absence of innervation. Lastly, histological methods relying on silver impregnation can be very difficult to interpret, and it is known that the intensity of the staining fades with time (D. Barker, personal communication).

Implications of the present findings for the mechanism of internal working of the spindle

Our results and those of Boyd *et al.* (1973) show that dynamic effects can occur with bag fibre contraction alone, and static effects with chain fibre contraction alone, but *not vice versa*. This, together with the fact that

although the distribution of static fibres is very often non-selective it is quite different from the distribution of dynamic fibres, seems to us to confirm the essential correctness of the mechanism of internal working of the spindle originally proposed by Matthews and his colleagues (Jansen & Matthews, 1962; Crowe & Matthews, 1964*a*; Brown & Matthews, 1966; Brown, 1971). The proposal is that slowly contracting bag fibres, whose tension can adjust only slowly to changes in length, cause the primary ending to have a large velocity sensitivity and also a high sensitivity to small movements, whereas chain fibres which contract quickly will tend to 'isolate' the afferent terminal from changes in length. These will be largely absorbed in the rapidly adjusting poles, so that velocity sensitivity and length sensitivity will be reduced.

The new feature which must be accommodated is the frequent concomitant bag contraction produced by static fusimotor fibres. Why does this not reveal itself in the output of the primary afferent terminal during stretching? Our present view is that the output from bag fibre terminals of the primary ending is swamped by the higher output from the chain terminals, the chain fibres contracting more quickly and powerfully. On this scheme the bag fibre terminations of static γ fibres appear at first sight quite redundant. One possibility is that the inevitable unloading generated in the central portions of bag fibres by chain fibre activation is partly taken up by the simultaneous bag contraction. 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.

At first sight it does appear surprising that during gallamine blockade bag fibre terminals of static fusimotor fibres do not succeed in making the spindle primary endings more dynamically sensitive, as it might have been expected that their effectiveness on chain fibres would have been more drastically reduced by the drug because in normal circumstances chain activation in part depends on propagated potentials. Instead our results provide additional evidence firstly for the effectiveness of non-propagated potentials in activating chain fibres, and secondly for the greater vulnerability of trail terminals relative to plate terminals to neuromuscular blockade. (This might simply arise from the smaller size of the trail terminals which might result in liberation of fewer transmitter quanta per impulse.) In fact the effect of post-synaptic fibre size on the size of junctional potentials (Katz & Thesleff, 1957) might render the static terminals on the large bag fibres more susceptible to early blockade than their terminals on chain fibres.

An additional possible reason for the failure to detect the static γ fibre's bag terminations by functional means may lie in the more central position-

ing of the trail terminals on the bag fibre compared with that of the dynamic fibre plates. Thus our failure to 'transform' static γ fibres into effective dynamic fibres during gallamine blockade need not be taken as evidence against the existence of non-selective innervation.

Implications of present findings for the development of motor innervation of the muscle spindle

There are at least two unusual features of the motor innervation of spindles. The first is that bag fibres appear to tolerate two sorts of functional motor nerve terminals upon them (and possibly even three if β fibres are taken into account). In this respect the bag fibre resembles a central neurone rather than a normal vertebrate muscle fibre, for even polyneuronally innervated muscle fibre types (e.g. in frogs, toads, fishes and birds) usually have endings from only one sort of motor fibre, while the specificity of mononeuronally innervated twitch fibres for motor nerves of a particular type in frogs and mammals is well known.

The second question that is raised concerns the mechanism by which static and dynamic fibres achieve the appropriate balance of terminals on each sort of intrafusal muscle fibre. Our evidence suggests that both types of γ fibre *can* innervate both sorts of intrafusal fibre, yet to achieve distinct static or dynamic actions, the two must predominantly innervate chain and bag fibres respectively. A clear-cut finding of completely selective innervation could be 'explained' by the same mechanisms which must exist to ensure specificity of central nervous connexions. Even a situation where dynamic fibres ended only on one particular region of bag fibres and static fusimotor fibres always ended on chains and also always on bag fibres, but in a different region, could be explained in the same way. Our findings are that the situation is more plastic, and the variability of the innervation pattern fits better with a graded system of some sort; either a variable affinity of the two sorts of intrafusal fibres for the different sorts of motor fibre, or a variable degree of competition to inactivate each other's terminals between static and dynamic fibres.

It is interesting that both frog spindles and mammalian eye muscle spindles, whose innervation is derived entirely from collateral branches of ordinary motor nerve fibres (Smith, 1964*a*, *b*; Barker, Harker, Stacey & Smith, 1972), paradoxically have a more selective innervation of their two types of intrafusal fibres. It could be that the tenuissimus and other small muscles are not typical of all hind-limb muscles. In other muscles non-selective innervation may be less common and the experiments of Brown, Goodwin & Matthews (1969) certainly provide quite strong evidence in favour of innervation sites on different intrafusal fibres for static and dynamic γ fibres of the cat soleus. Yet other possibilities to be borne in

mind are the age and state of 'fitness' of the cats. Our cats are laboratory bred and have mostly been a few months old. Rejection of polyneuronal innervation in rat and kitten extrafusal fibres only occurs some time after birth (Redfern, 1970; Bagust, Lewis & Westerman, 1973), and it may be that the comparable 'sharpening up' process occurs later inside the spindles and it may moreover depend on the physical state of the animal. We do not, however, consider that age is of fundamental importance, for we have lately deliberately looked at the distribution of static fibres in a cat weighing over 3·0 kg, and we found both bag and chain glycogen depletion. Lastly, the γ fibres we have studied have all been of relatively high conduction velocity, and the possibility exists that the small γ fibres, most of which are probably static γ fibres (Brown *et al.* 1965) are specific in their termination sites. Jones (1966) finds this to be the case in the Australian possum.

The presence of extensive non-selective innervation also seems to cast doubt on the recent suggestion of Milburn (1973) that it is the γ fibres which determine the myofibrillar and enzymatic differences between bag and chain fibres.

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EXPLANATION OF PLATES

The length calibration bars are 25 μm. All sections cut at 8 μm.

PLATE 1

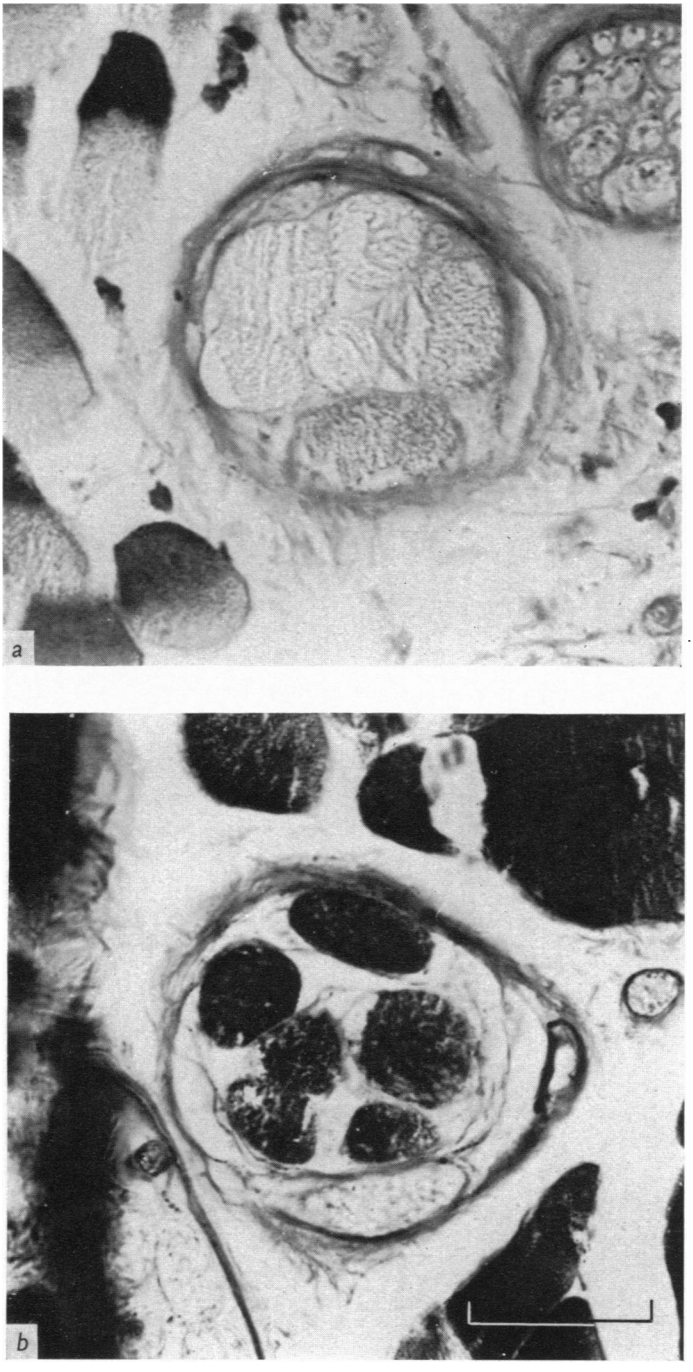
To illustrate the effect of whole nerve stimulation, at strengths supramaximal for γ fibres, on spindle glycogen content. *a*, Experimental muscle; all intrafusal fibres stain very little and therefore contain very little glycogen; *b*, control muscle from other leg showing standard dark staining indicating abundant glycogen.

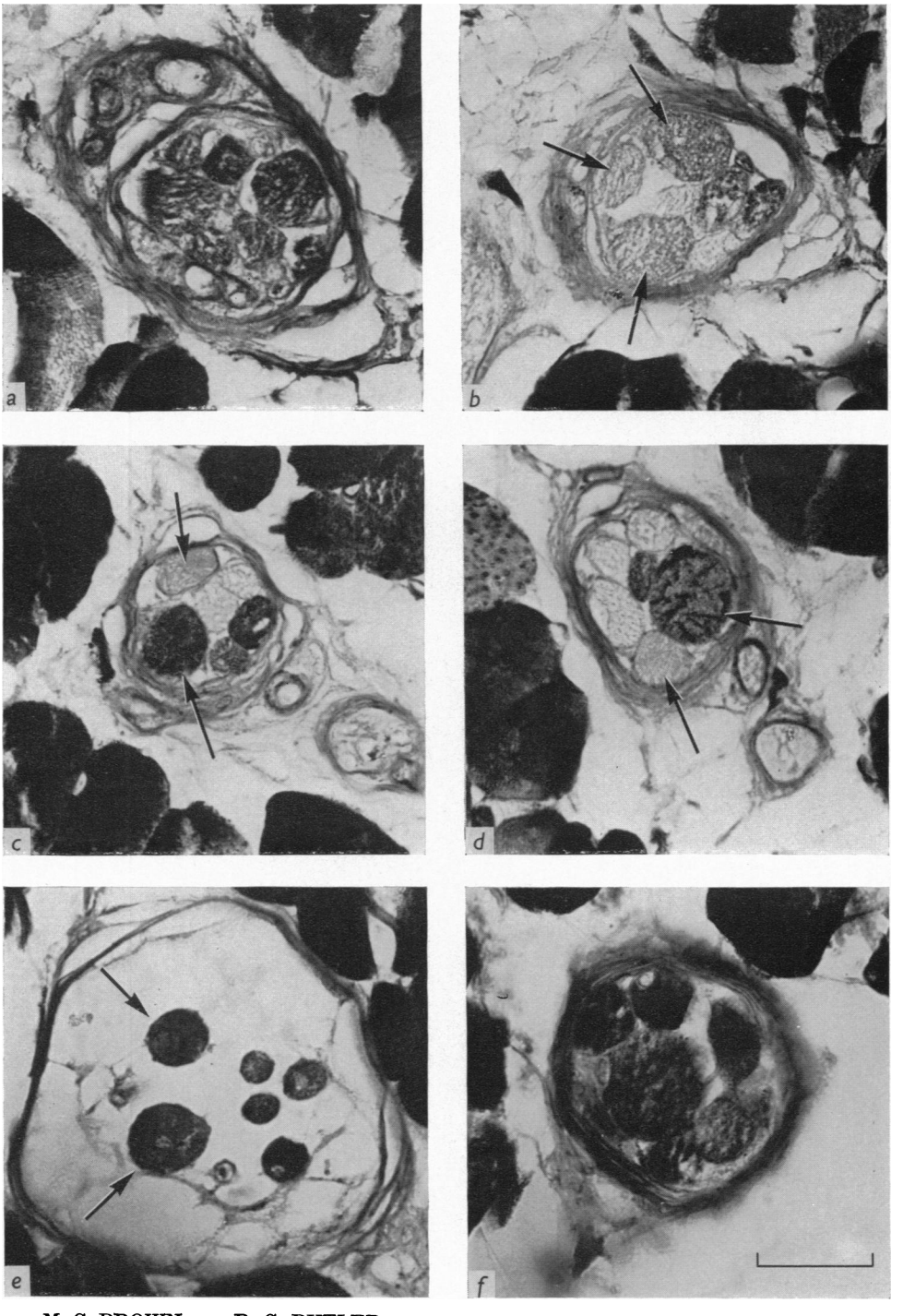
PLATE 2

To illustrate effects of stimulating a single static γ fibre on spindle glycogen content. *a*, *b*, From the two poles of one spindle showing no glycogen depletion at one pole (*a*), and at other pole (*b*) two depleted chain fibres, two undepleted chain fibres, and three paler than normal bag fibres. *c*, *d*, *e* From another spindle activated by the same static γ fibre. *c*, One pole showing two pale chain fibres and one pale bag fibre. *d*, Other pole showing three pale chain fibres and one pale bag fibre. *e*, Juxta-equatorial region of this spindle with no depletion. *f*, Spindle from control muscle. In *b*, *c*, *d*, *e*, bag fibres are indicated by arrows.

PLATE 3

To illustrate effect of stimulating three static γ fibres simultaneously on spindle glycogen content. *a*, Experimental spindle showing pale staining (very little glycogen) in all intrafusal fibres. *b*, Control spindle from tenuissimus of other leg.


a
b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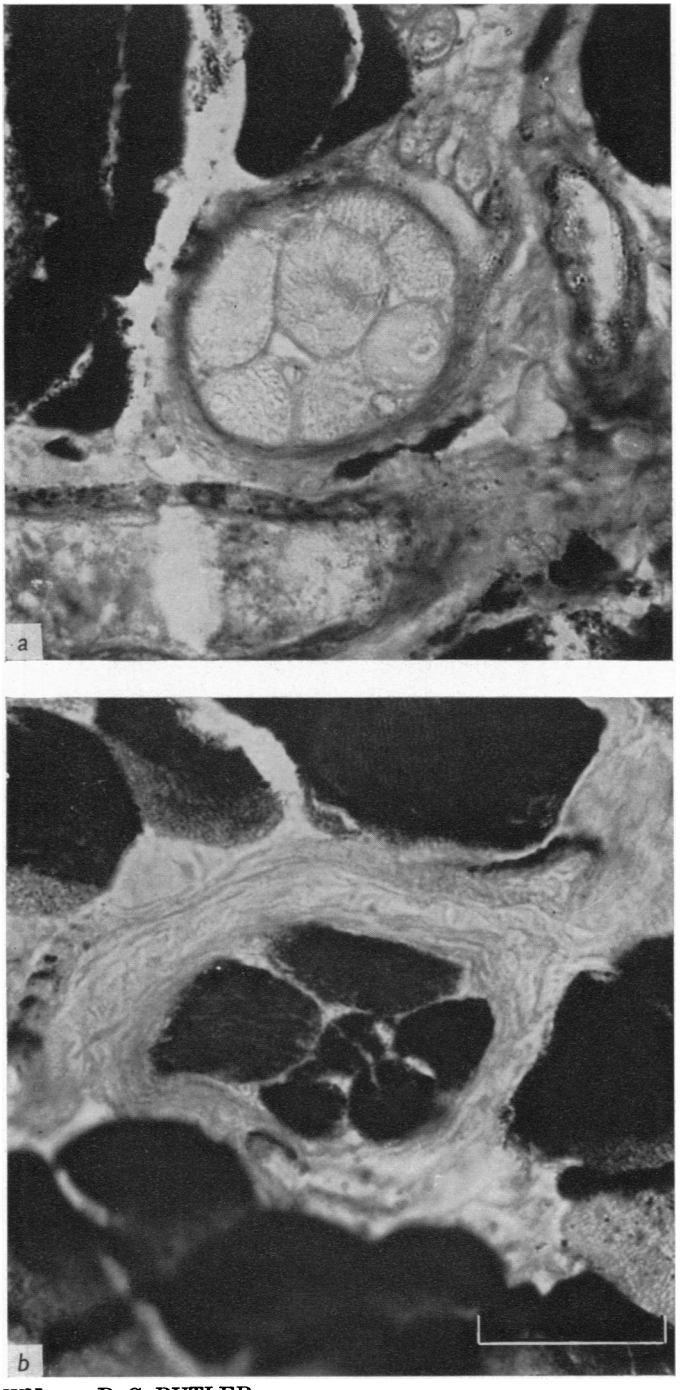
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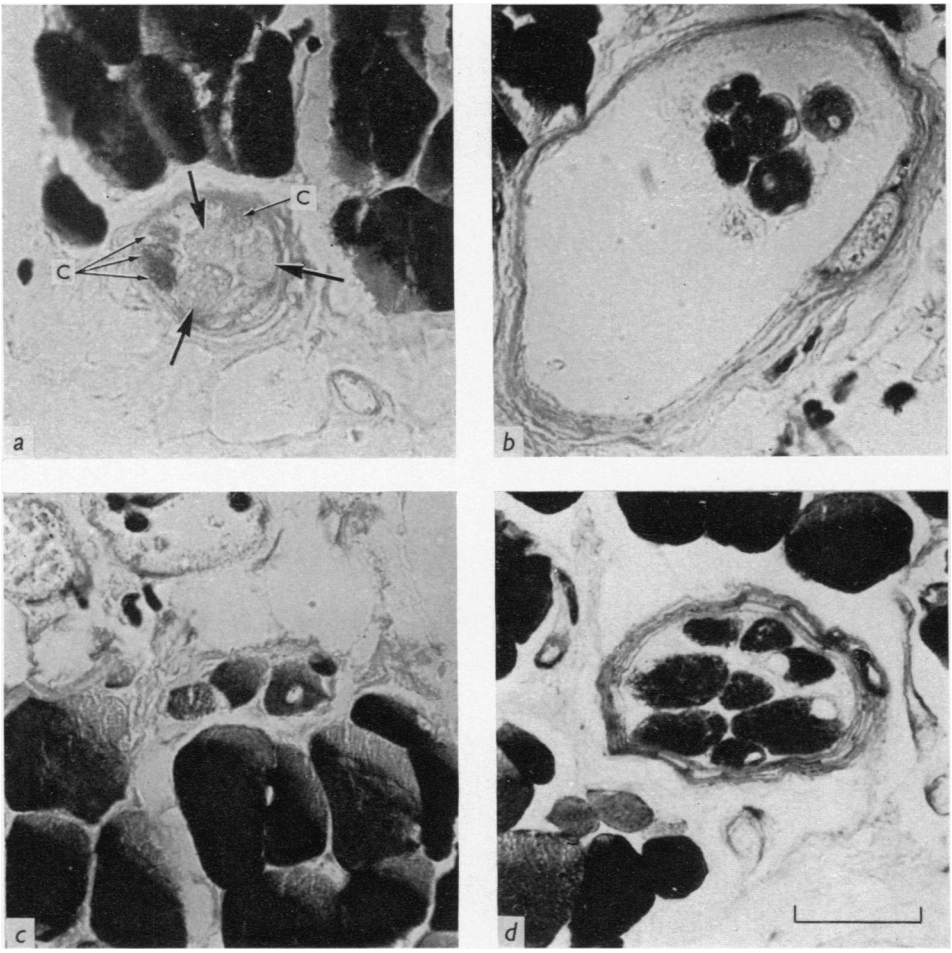
C
C
a
b
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PLATE 4

To illustrate effect of stimulating a single dynamic γ fibre on spindle glycogen content. *a*, *b*, *c*, Sections taken at different regions from one pole of affected spindle. *b*, Juxta-equatorial region – no depletion; *c*, extracapsular region – no depletion; *a*, intracapsular polar region showing three pale staining bag fibres, and four chain fibres, one of which is certainly paler than normal, two of which are normal, and one possibly depleted. *d*, Spindle from control tenuissimus. In *a*, large arrows indicate bag fibres; C indicates chain fibres.