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OBSERVATIONS ON THE NUMBER OF NUCLEI WITHIN THE FIBRES OF SOME RED AND WHITE MUSCLES

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SUMMARY

Nuclei have been enumerated in muscle fibres of different physiological properties within adult rats and rabbits. Almost invariably, and regardless of muscle type, there is a direct relationship between the cross-sectional area (or fibre breadth^2) of muscle fibres and the number of nuclei within them. The one exception occurred in muscles of older rats where increased nuclear numbers do not always appear to result in broader muscle fibres.

The greater complement of nuclei in broader fibres is accompanied by larger amounts of cell substance per nucleus.

Confirming early observations in the literature, red fibres of the slow-phasic type have more nuclei than have white, fast-phasic fibres of similar breadth. These conclusions are not vitiated by differences in the number of nuclei within capillaries or in satellite cells, by differences in nuclear length or by variation in the degree to which fibres are contracted.

In respect of their complement of nuclei, and the average amount of cell substance formed per nucleus the small red fibres that occur within muscles of predominantly fast-phasic character appear to be fast- rather than slow-phasic in type.

When the number of nuclei observed per fibre is plotted against fibre cross-sectional area, the shapes of the resulting distributions suggest that estimates of muscle nuclei may be valuable not only as an index of growth potential, but of the extent to which that potential is expressed. In one muscle, the above distribution was of a form which indicated that some fibres may have formed abnormally large amounts of protein per nucleus. However, this was not adequately confirmed.

Various factors have been investigated that are relevant to the accuracy of enumerating nuclei and measuring fibre breadths.

INTRODUCTION

It is tempting to suppose that the number of nuclei within a muscle fibre is some measure of the maximum speed and extent of growth that the fibre can express in favourable circumstances. However, proving whether this is so presents some difficulties. Although muscle fibres acquire more nuclei as they grow, this process is possibly a fortuitous accompaniment to some other growth-regulatory mechanism. Relationships between nuclear numbers and muscle growth are also potentially complicated by nuclear replication in response to trauma, given that muscle nuclei can increase in number as a result of disease (cf. Schiefferdecker, 1909; Landing, Dixon & Wells, 1974), surgically induced denervation and physical damage (Teräväinen, 1970; Hanzlíková, Macková & Hník, 1975; Ontell, 1975).

Skeletal muscles contain fibres of different biochemical and contractile properties.

One must therefore have regard for 2 possibilities: that different numbers of nuclei are required to produce the same amount of growth within different kinds of fibre, and that nuclear numbers can be influenced by stresses associated with different patterns of contractile activity.

For example, biochemists have found red, slow-phasic muscles to possess somewhat elevated amounts of DNA and RNA per unit weight of muscle (reviewed Young, 1970; Burleigh, 1974). Several early microscopists also observed that such muscles in the rabbit exhibit more nuclei per unit of cross-sectional area of fibre than do white muscles of the fast-phasic sort (Ranvier, 1874*a*; Schiefferdecker, 1909, 1928; Hoffmann, 1938; Watzka, 1939). Some of the evidence suggests that red fibres can attain comparatively large breadths. Schiefferdecker (1909, 1928) also found that within muscles, broader fibres tend to have more nuclei and that they frequently possess more cell substance per nucleus. Landing *et al.* (1974) have obtained similar results on humans.

Red fibres possess particularly abundant capillaries (Ranvier, 1874*b*) that often indent the fibre surface. It is possible that the nuclei of capillaries were included to some extent in the enumerations of early microscopists and that they are contributing to biochemical determinations of nucleic acids. This argued for a re-examination of the early microscopy using sections of muscle fibres that are thin enough to allow the resolution of closely-apposed nuclei in capillaries and satellite cells (see Venable, 1966*a*, *b*).

In addition, it now appears that not all red fibres contract slowly. Most muscles contain a proportion of small fibres that, on histological evidence, appear to be fast-phasic (Gauthier & Padykula, 1966; Peter, Barnard, Edgerton, Gillespie & Stempel, 1972). They occur in association with broader, fast-phasic fibres that in certain circumstances are visibly whiter. In the semitendinosus of the rat, narrow red fibres and broader, white fibres are concentrated in bands, one red and one white (Gauthier, 1969). This muscle seemed to merit study.

MATERIALS AND METHODS

Female Norwegian rats and New Zealand rabbits were obtained locally, unless stated otherwise. Unanaesthetized rabbits were killed by a captive bolt pistol and rats under ether anaesthesia were killed by cervical dislocation.

Only muscles from right legs were used. Muscles of the rat were fixed while still attached to their origins and insertions. Those of the rabbit were dissected free and fixed while wired under moderate tension to a splint.

When nuclei were to be estimated in transverse sections of muscle fibres, or to be examined in the electron microscope, the muscles were fixed in 4 % glutaraldehyde for a total of 4 h at room temperature. Postfixation was in 1 % OsO_4 for 40–50 min at room temperature and cacodylate buffer (0·1 M, pH 7·2) was used throughout. The fixed muscle was embedded in Epon (Luft, 1961), each strip being cut transversely and arranged at right angles in embedding trays. Serial sections (0·7 μm) were cut with a Huxley Model 1 ultramicrotome, mounted on coverglasses and stained at 60 ° C with 1 % toluidine blue in aqueous (1 %) borax.

Nuclei were enumerated in transverse sections of non-oblique, well-fixed fibres whose breadths were measured with an eyepiece micrometer. Fibres were discounted if the ratio of the maximum breadth to that in a direction normal to it exceeded 1·30. The mean of these two measurements was taken as the fibre breadth. Satellite cells and closely apposed interstitial cells

could be recognized under the light microscope by the gap between them and the muscle fibre, and by their comparatively dense chromatin (see Venable, 1966*a*, *b* and Fig. 1, p. 272). They were excluded from the counts.

Sections were routinely viewed using the 1·32 NA oil-immersion objective of a Leitz Ortholux microscope and a yellow filter, nuclear and sarcomere lengths being measured with a drawing device. Electron microscopy was performed with an AEI EM6B microscope on unsupported sections that had been stained with uranyl acetate followed by lead citrate.

To estimate nuclei in longitudinally oriented fibre segments, the latter were isolated by collagenase digestion according to Kopriwa & Moss (1971), except that the buffer was 0·04 M phosphate (pH 6·8) containing 0·2 M sucrose. In the minority of cases, the sucrose was omitted and control measurements showed that fibre breadths were unaffected. At least 10 randomly selected fragments (of breadths 0·5–1·0 mm approximately) were taken from each muscle. The isolated fibres were incubated for 50 min in ice-cold 1 % glutaraldehyde in the same buffer. This inhibited residual collagenase and proteolytic activity. After being washed, the fibres were stained with Gallamine blue (Dutt, 1974), dehydrated, infiltrated with Epon, and mounted on coverslips which were inverted over a drop of Epon in a slide chamber. The Epon was then polymerized.

Nuclei were enumerated in visually intact regions of the fibres using a 25 × objective and a drawing device. The latter was also employed to measure sarcomere lengths and fibre breadths. In enumerating nuclei, 2 lines were drawn across the fibre at the required distance apart and at right angles to the fibre's longitudinal axis. By focusing through the segment, all nuclei between the lines were counted including those that touched or overlapped one of the lines; those that impinged on the other were omitted. The enumerations thus become independent of nuclear length since there will be equal variation in the probability of nuclei impinging on each line and being respectively counted or omitted.

To estimate cytochrome a and total haem, muscles were homogenized in ice-cold 0·15 M KCl with a pestle homogenizer. After centrifuging at 30000 **g** for 20 min the procedure of Cheah (1970) was used to estimate total haem in the supernatant and cytochrome a in the reconstituted pellet.

Collagenase was obtained from Sigma and Gallamine blue from Difco.

Abbreviations. RTRST, RTWST, RTBF and RTSL respectively represent the red and white portions of the semitendinosus, the biceps femoris and the soleus of the rat; RBST and RBAD represent the semitendinosus and the adductor magnus of the rabbit. In this paper, the semitendinosus of the rabbit is taken to be the red muscle in the thigh, that muscle being encased in a much larger white muscle, the adductor magnus.

RESULTS

Results on transverse sections

In Epon-embedded sections (1 μm or less in thickness) of muscle, the nuclei of capillaries and satellite cells can be distinguished from sub-sarcolemmal nuclei, i.e. nuclei that occur beneath both the plasma membrane and the basal lamina of the fibres (Venable, 1966*a*, *b*). Satellite cells are also found beneath the basal lamina of a muscle fibre but are outside the plasma membrane (cf. Venable, 1966*a*, *b*; Muir, Kanji & Allbrook, 1965; Figs. 1, 2).

Table 1 shows that the number of nuclei seen per fibre is greater in broader fibres, regardless of muscle type. The correlation is invariably significant statistically at the 5 % level or less and it has also been observed in another white muscle of the rat, the biceps femoris (results not shown). Table 1 also indicates that in both species of animal, the number of nuclei within slow-phasic fibres is greater than the number within fast-phasic or presumptive fast-phasic fibres of similar breadth.

As regards the fast-phasic or presumptive fast-phasic muscles shown in Table 1,

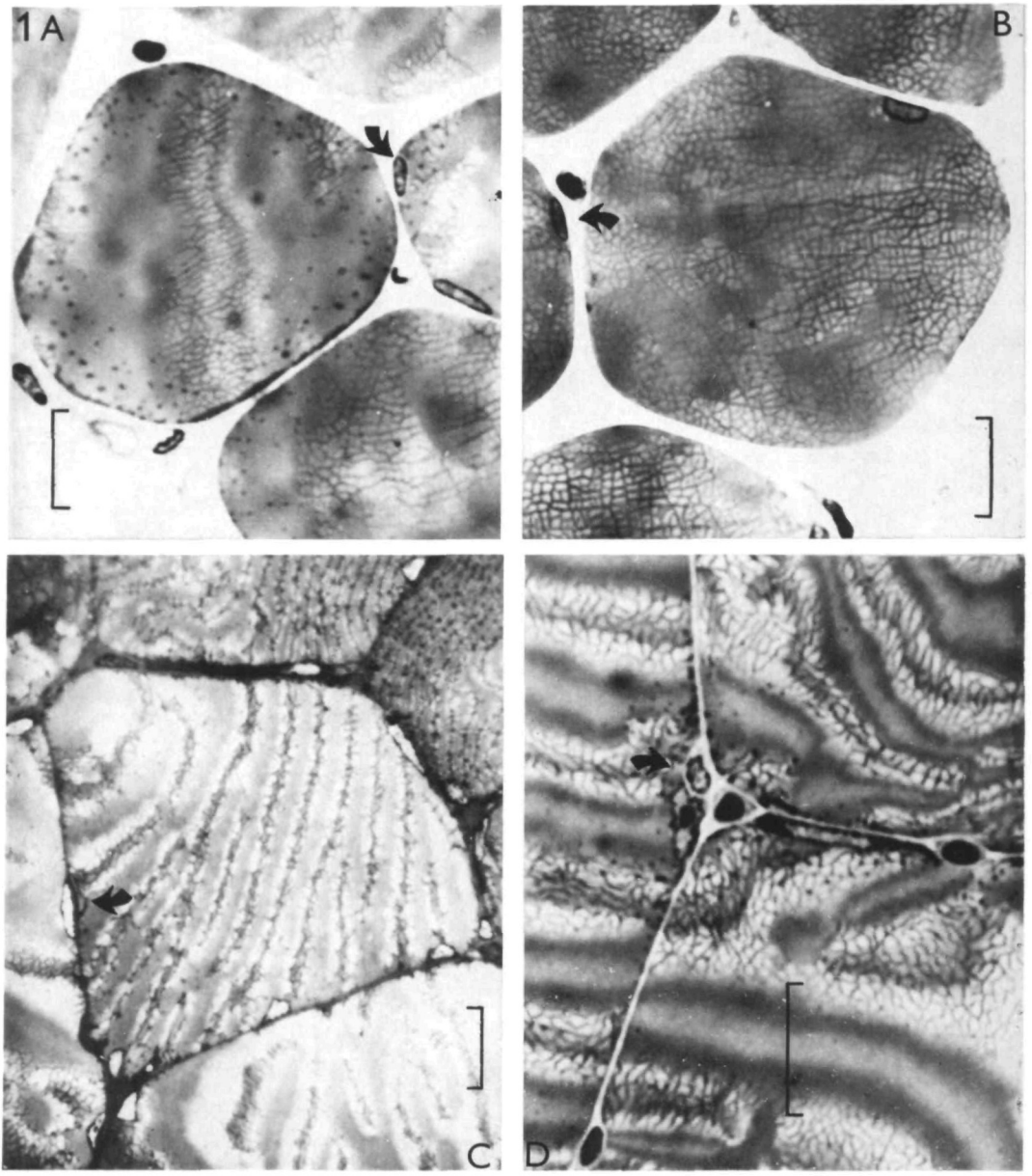

Fig. 1. All scale lines represent 10 μm. A, red fibre of rat semitendinosus showing no nuclei. A satellite cell (arrowed) and a subsarcolemmal nucleus are shown in the adjacent fibre.

B, white fibre of rat semitendinosus, exhibiting one subsarcolemmal nucleus. Closely apposed to the adjacent fibre is a satellite cell (arrowed).

C, a fibre from rabbit semitendinosus at a lower magnification. The fibre has 4 prominent subsarcolemmal nuclei and 1 dark-staining nucleus (arrowed).

D, detail from the rat soleus showing a prominent satellite cell (arrowed) adjacent to a subsarcolemmal nucleus.

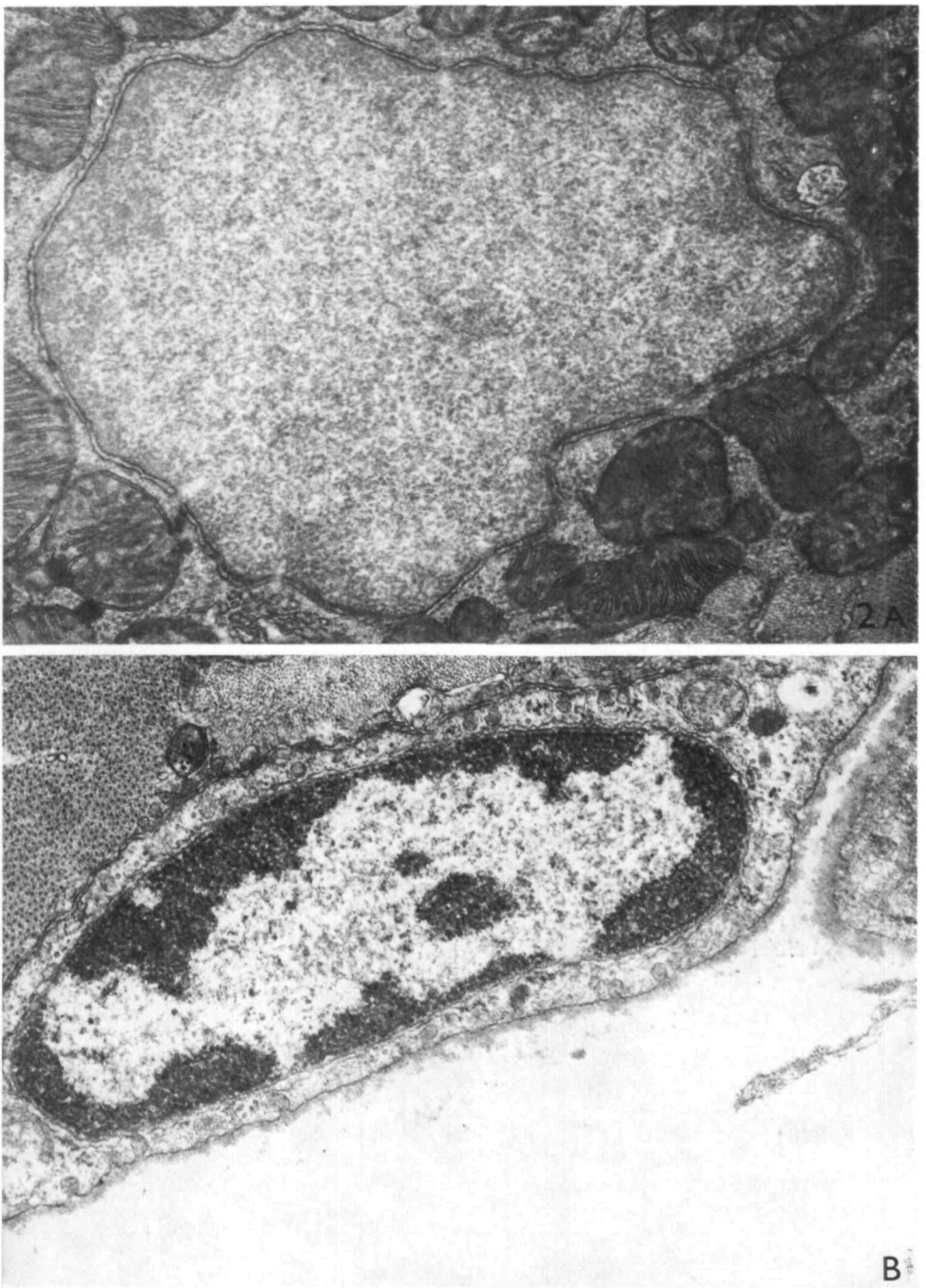

Fig. 2. A, nucleus within a fibre of rat soleus. The nucleus is near the periphery of the fibre and is surrounded by accumulations of mitochondria. × 30000.

B, satellite cell of rat semitendinosus. A pair of plasma membranes can be seen separating the nucleus of the satellite cell from the substance of the muscle fibre. × 30000.

the adductor magnus of the rabbit tends to have the broadest fibres with the most nuclei. Although it contains high concentrations of mitochondria (measured by cytochrome a) and soluble haem-containing pigment (Table 2), the red portion of the semitendinosus of rats has fibres that do not display the elevated concentrations of

Table 1. *Numbers of nuclei observed in transverse sections of muscle fibres of different breadth*

Muscle	Fibre breadth category, μm	Mean no. of nuclei seen per fibre in transverse section	Sarcomere length, μm
RTRST	0–27·5	NM	NM
(F)	27·6–47·5	0·87 (92)	2·63
	47·6–67·5	1·25 (38)	2·51
	67·6–87·5	2·56 (3)	2·53
	44.8	**1·02**	**2·59**
RTWST	0–27·5	0·50 (6)	2·69
(F)	27·6–47·5	0·91 (57)	2·72
	47·6–67·5	1·36 (85)	2·58
	67·6–87·5	1·40 (15)	2·43
	51·8	**1·19**	**2·62**
RBAD	0–27·5	0·46 (28)	2·60
(F)	27·6–47·5	0·90 (63)	2·61
	47·6–67·5	1·43 (110)	2·64
	67·6–87·5	2·48 (62)	2·52
	55·2	**1·45**	**2·60**
RTSL	0–27·5	NM	NM
(S)	27·6–47·5	1·50 (12)	2·77
	47·6–67·5	2·35 (127)	2·92
	67·6–87·5	2·88 (8)	2·84
	56·4	**2·31**	**2·90**
RBST	0–27·5	NM	NM
(S)	27·6–47·5	1·33 (3)	2·91
	47·6–67·5	3·31 (31)	2·71
	67·6–87·5	3·86 (59)	2·67
	⩾87·6	4·42 (14)	2·95
	72·0	**3·70**	**2·72**

NM: not measured because an insufficient number of fibres occurred in that category.
In parentheses are the total number of fibres examined, those for any given muscle being pooled from 5 animals. Mean values per entire muscle are given in bold type. F: fast or presumptive fast-phasic; S: slow-phasic.

nuclei found in the red, slow-phasic muscles. Rather the complement of nuclei in the narrow fibres of this muscle is essentially identical to that of corresponding fibres in the 2 white (fast-phasic) muscles that have been examined (Table 1).

The above estimates of nuclei are not confused by the presence of satellite cells. As determined by light and electron microscopy, such cells accounted for 8% or less of the fibre nuclei in each muscle, 100 nuclei and upwards being examined in each case.

Other authors have obtained similar percentages (Venable, 1966*a*, *b*; Muir *et al.* 1965; Allbrook, Han & Hellmuth, 1971).

In Table 1, the number of very narrow ($\leqslant 27{\cdot}5$ μm) fibres in the adductor magnus includes some tapering fibres that terminate within fasciculi. This was confirmed from serial sections. Consequently, Table 1 indicates that tapering fibres have progressively fewer nuclei towards their ends. They also possess less cell substance per nucleus since the mean ratio of fibre cross-sectional area to nuclear numbers in this category of fibre was only 440 μm^2. The mean value for the adductor magnus as a whole is 1675 (Table 3).

Table 2. *Concentrations of cytochrome a and soluble haem in muscles of adult rats and rabbits*

Muscle	Cyt. a, nmol/g wet muscle	Total haem, nmol/g wet muscle
Soleus (rat)	$12{\cdot}4 \pm 2{\cdot}9$	$82{\cdot}4 \pm 9{\cdot}7$
Red semitend. (rat)	$11{\cdot}3 \pm 0{\cdot}4$	$51{\cdot}5 \pm 9{\cdot}7$
Semitend. (rabbit)	$4{\cdot}7 \pm 0{\cdot}9$	$54{\cdot}0 \pm 5{\cdot}8$
White semitend. (rat)	$4{\cdot}1 \pm 0{\cdot}8$	$13{\cdot}6 \pm 1{\cdot}5$
Add. magnus (rabbit)	$1{\cdot}9 \pm 0{\cdot}8$	$3{\cdot}2 \pm 0{\cdot}2$

Mean values are given with standard deviations. The ages and weights of the animals were in the range quoted in Table 1.

Results on rabbit muscles are the mean of values determined on 3–4 individual muscles. The results for the rat are the means of estimates on 3–4 groups of each muscle, each group being pooled from 3 animals.

Table 3 summarizes the ratios of fibre cross-sectional area to nuclear number in each adult muscle. Some data on the biceps femoris of rats are included. These particular animals were of a larger strain (body weight 375–411 g; age 7–8·5 months) than was used in compiling Table 1 (body weight 240–260 g; age 4·5–7 months). In this muscle, the mean breadth of fibre (62·4 μm) was the largest of the fast-phasic muscles studied, showing that rats are, in principle, capable of producing muscle fibres that are as broad as or are broader than those of the rabbit.

Table 3 shows that within each group of fast-phasic or slow-phasic muscles, the mean ratio of fibre cross-sectional area to the number of nuclei seen in transverse section varies by comparatively little (18% or less). However, allowing for the effects of variable nuclear length could increase these differences further by up to 20%.

In Table 3, fibres have also been classified according to whether their breadths are greater or less than values near the mean for each muscle. The consistent observation is for the ratio of fibre cross-sectional area to nuclear number to be greater in broader fibres, despite a tendency for nuclei to be slightly longer in such fibres (unpublished) thus increasing the chances of detecting the nuclei in transverse section.

There is considerable variation in the number of nuclei that can potentially be seen in a sectioned fibre of given breadth. This is illustrated in Fig. 3A–D in which the number of nuclei observed per fibre is plotted against the square of fibre diameter, the latter parameter being proportional to fibre cross-sectional area. Given that the

broader fibres within a muscle possess comparatively large amounts of cell substance per nucleus, then one might expect the regression lines that best fit the data should be curves whose slopes progressively diminish at higher cross-sectional areas of fibre. In Fig. 3 A–D the least squares method has been used in an attempt to fit the data by such curves of the form $a+be^{-kx^2}$, where x = fibre breadth and y = nuclear number. However, the computer-generated lines (dashed lines) that were obtained did not significantly differ from linearity.

Table 3. *Showing range of muscle fibre breadth, cross-sectional area of fibre per nucleus and mean nuclear length in representative muscles*

Muscle	Range of fibre breadth, μm	Cross-sectional area of fibre per nucleus, μm^2	Mean nuclear length, μm
RTRST	$\leqslant$47·5	1448	
	>47·5	1720	
		1545	**15·5**
RTWST	$\leqslant$47·5	1425	
	>47·5	2036	
		1755	**18·4**
RBAD	$\leqslant$47·5	1125	
	>47·5	1845	
		1675	**19·5**
RTBF	$\leqslant$65·0	1529	
	>65·0	1946	
		1809	**18·5**
RTSL	$\leqslant$55·0	974	
	>55·0	1211	
		1086	**14·1**
RBST	$\leqslant$70·0	906	
	>70·0	1311	
		1118	**14·7**

Mean values per entire muscle are given in bold type.

Fig. 3 suggests that this is not caused by random variation within the data, but by the manner in which the points are distributed. In 3 of the muscles there is a quite marked relationship between nuclear number and the minimum cross-sectional area of fibre that contains a given number of nuclei (Mr H. J. MacFie pointed this out to me). It is delineated by the solid lines which represent computer-generated logistic fits between numbers of nuclei and the corresponding minimum cross-sectional areas of fibre (i.e. the data were fitted to segments of curves whose general formula is $y = a+c/1+e^{-b(x-m)}$). The lines are rather strongly curved in the case of the rabbit semitendinosus and the pooled red and white regions of the rat semitendinosus. The fit is less certain in the case of the rat soleus, possibly because of 2 spurious points.

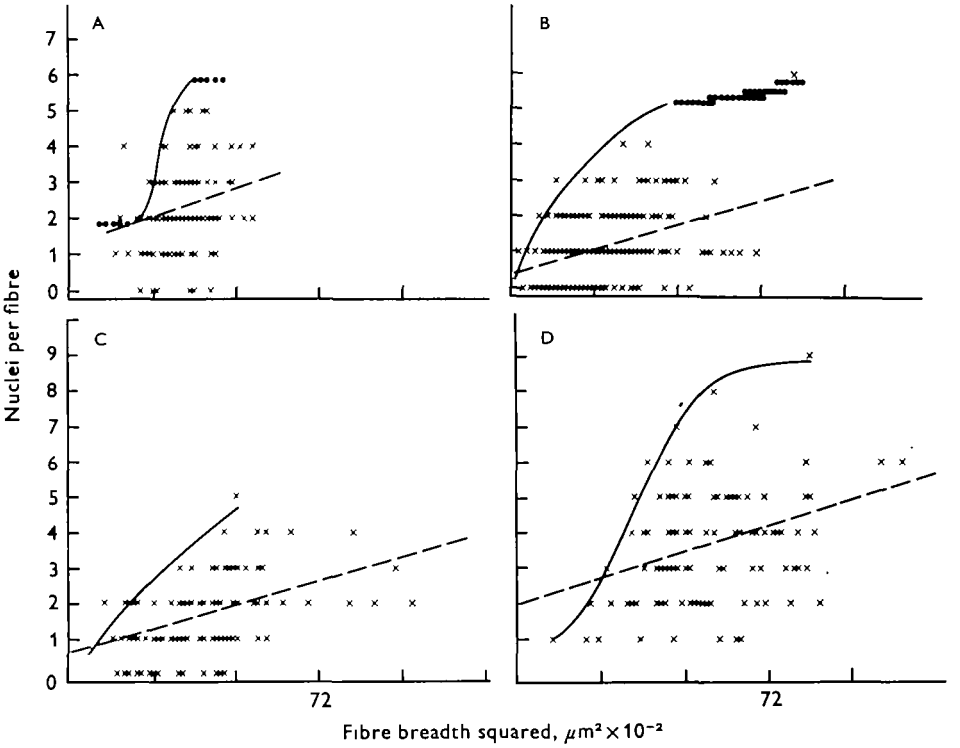

Fig. 3. No. of nuclei observed/fibre in transverse section against (fibre breadth)^2. The dashed line represents the best overall correlation between nuclear numbers and (fibre breadth)^2, as determined by the method of least squares. The solid line is the optimum fit between nuclear numbers and the minimum (breadth)^2 of fibre containing a given number of nuclei (see text). The solid circles represent regions of uncertain fit. A, rat soleus (RTSL); B, pooled red and white regions of the rat semitendinosus (RTST); C, rabbit adductor (RBAD); and D, rabbit semitendinosus (RBST).

Results on longitudinally oriented fibres

Nuclei were also estimated in segments of isolated fibres by a procedure which should be independent of nuclear length (see Methods). Only muscles of the fast-phasic or presumptive fast-phasic type were investigated (i) because of the greater ease with which their fibres are separated with collagenase and (ii) because fewer capillary nuclei are present.

Nuclei were enumerated in fibre segments that were either 50 sarcomeres or 100 μm in length. Capillary nuclei were excluded. They accounted for less than 8% of the total nuclei but the preservation of internal detail did not suffice to determine the percentage of satellite cells that were present. The previous measurements on transverse sections indicated that both portions of the rat semitendinosus have narrower fibres with fewer nuclei than those of the adductor magnus of the rabbit (Table 1). Although near their final weight, the rats were chronologically younger (age 4·5–7 months; wt 240–260 g) than the rabbits (9–13 months; 3·7–4·2 kg) and one cannot say whether the respective fibres have inherently different potentials for growth.

Nuclei were therefore enumerated in fibres isolated from the entire semitendinosus muscle of rats aged approximately 5 and 12 months and from the adductor magnus of rabbits aged 11 months. The results are summarized in Table 4.

There is again a tendency for the rabbit fibres to be broader and to have more (over 20% more) nuclei than the fibres of the rat semitendinosus at 5 months. However, mean numbers of nuclei in the fibres of 11-month rabbits and year-old rats were

Table 4. *Mean breadths, sarcomere lengths and nuclear complements of fibres isolated from the semitendinosus of rats and the adductor magnus of rabbits*

Animal and muscle	Nuclei/50 sarcomeres	Nuclei/100 μm length of fibre	Mean fibre breadth, μm	Mean sarcomere length, μm
ST of female rats (20–23 wk old; 247–265 g)	$9{\cdot}14^{1}$ (4)	$8{\cdot}60^{1}$	$31{\cdot}54^{1}$	2·16
ST of female rats (48–55 wk old; 260–341 g)	$10{\cdot}70^{2,a}$ (5)	$10{\cdot}05^{2}$	$36{\cdot}60^{1}$	2·21
AD of female rabbits (10–11 m old; 4·0–4·2 kg)	$11{\cdot}20^{b}$ (4)	$11{\cdot}80^{3}$	$46{\cdot}97^{3}$	1·89

In parentheses are the number of preparations studied; measurements were made on at least 40 fibre segments in each preparation. Numbers with different numerical superscripts in any vertical column are significantly different at the 1% level; those with different letters as superscript are just significantly different at the 5% level. ST, semitendinosus; AD, adductor magnus.

respectively 11·20 and 10·70 per 50 sarcomeres, a difference of 5% which was only just significant. In the older rats, the mean fibre breadth was still quite markedly (about 22%) less than that of the rabbit adductor. Although these rats were of similar chronological age to the rabbits, processes associated with senescence are perhaps more advanced in the rat to the point of impairing the growth-promoting potential of muscle nuclei.

Table 4 also indicates that when nuclei were related to unit length (100 μm) of fibre, differences between the rabbit and rat muscle at both ages were accentuated. For example, rabbit fibres (of mean sarcomere length 1·89 μm) exhibited an average of 11·80 nuclei per 100 μm length of fibre. The corresponding value was 10·05 in the semitendinosus muscle of the older rats (mean sarcomere length 2·21 μm), and this number of nuclei was significantly different from that in the rabbit. Therefore, apparent differences in nuclear numbers can be influenced by the method of measurement.

Confirming the results from transverse sections, highly significant ($P \leqslant 0{\cdot}001$) correlations were obtained between nuclear numbers and fibre cross-sectional areas in the semitendinosus and adductor magnus muscles of rats and rabbits respectively. This

was so whether nuclei were enumerated in fibre segments that were 50 sarcomeres (dashed lines in Fig. 4A–C) or 100 μm long (unpublished).

The broader fibres in isolated populations also possess more cell substance per nucleus: If one divides the fibres from the rat semitendinosus at 5 months into categories whose mean (radius)^2 is respectively greater or less than 300 μm^2, the mean volume of cell substance per nucleus is 16000 μm^3 in the broader category of fibre compared

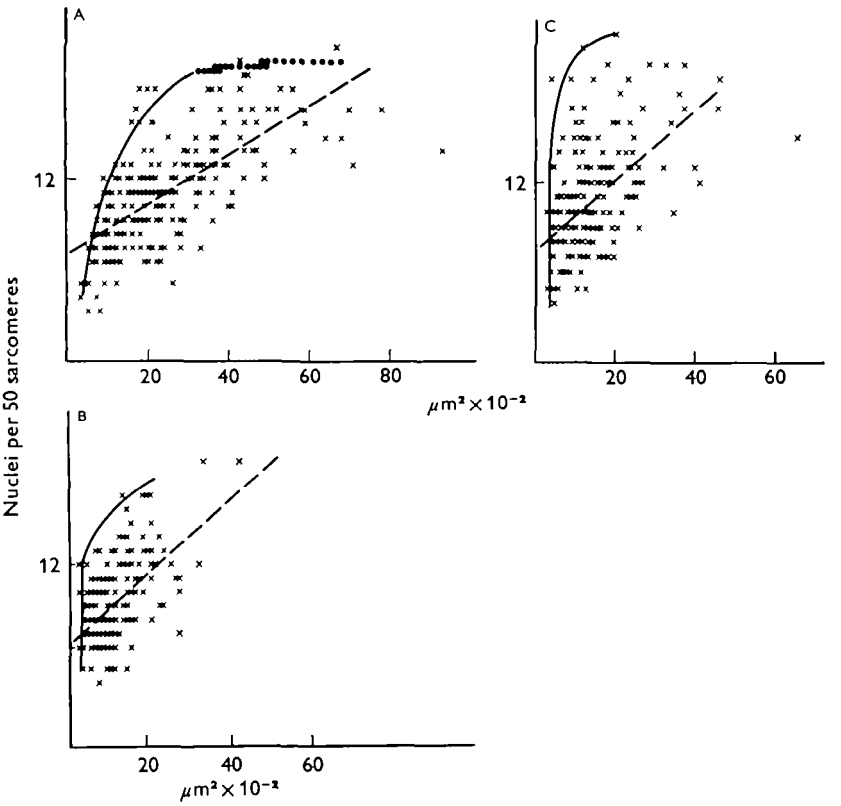

Fig. 4. No. of nuclei in longitudinally oriented fibre segments against (fibre breadth)^2. The dashed and solid lines and the solid circles have the meanings given in Fig. 3, with the exceptions stated in the text. A, rabbit adductor; B, rat semitendinosus at approx. 5 months; C, rat semitendinosus at 12 months.

with 7800 μm^3 in the narrower group. For fibres of mean (radius)^2 greater or less than 500 μm^2 in the rabbit adductor, the corresponding values are respectively 17200 and 11300 μm^3. In both muscles, this difference between broader and narrower fibres is highly significant ($P \leqslant 0{\cdot}001$).

Compared with transverse sections, the left-hand edge of the distribution of fibre cross-sectional areas (fibre breadth^2) against nuclear number appeared even more vertical in the case of isolated fibres. As regards the rabbit adductor, the edge is delineated as part of a logistic curve as before (Fig. 4A). In Fig. 4B, C (the rat semitendinosus at 5 and 12 months respectively), it is indicated manually since the nearly vertical trends over the lower part of the range do not fit a curve of the above form.

DISCUSSION

Ultimately, this work aims to establish whether increased numbers of myogenic nuclei in muscle fibres can arise from accelerated cell replication and can subsequently accelerate growth of the muscle fibres. Rates of growth were not studied in the present work but the results indicate that in the rabbit and the rat, the number of nuclei within muscle fibres is directly related to the cross-sectional area of fibre that is eventually achieved. This is so regardless of whether the muscles are slow- or fast-phasic in type. The observations on the rabbit confirm others obtained before 1930 and indicate that the older enumerations were not vitiated by the presence of satellite and capillary nuclei and by differing extents of fibre contraction.

Fibres in red, slow-phasic muscles possess more nuclei than do fibres of similar breadth in fast-phasic muscles. However, despite their high capacity for mitochondrial respiration, the narrow red fibres of the rat semitendinosus have relatively few nuclei, numbers of the latter being virtually identical to those within narrow fibres of fast-phasic muscles (Table 1). Schiefferdecker (1909, 1928) made a similar observation on the carp. Thus, the number of nuclei in narrow red fibres appears related to the extent of growth of the fibres and not to their physiological properties. However, the muscle with the broadest fibres that contained most nuclei (the semitendinosus of the rabbit) showed occasional evidence of covert pathological events i.e. nuclei occurring in rows, abnormally prominent protuberances of cytoplasm at the fibre periphery and infiltration of fibre bundles by fat cells. An effect of trauma on nuclear numbers cannot wholly be excluded therefore (see Introduction). In addition, incipient old age in rats possibly impairs the ability of nuclei to sponsor muscle growth (see Results section).

There are theoretical grounds for believing that in the course of accumulating a given quantity of protein, fibres with elevated numbers of nuclei may use less energy in resynthesizing degraded protein, i.e. in one sense their growth should be more efficient, although the fibres should be physiologically immature in terms of their specialized contractile properties (Burleigh, 1976). In the present work, the most marked differences in mean nuclear concentration have been observed in slow-phasic relative to fast-phasic fibres. The former display some indications of immaturity in that they possess low amounts of cell substances per nucleus, the amount and activity of mitochondrial respiratory enzymes is high and the activity of phosphorylase and glycolytic enzymes is low (cf. Table 2 and Burleigh & Schimke, 1969; Burleigh, 1974). Perry (1970) has suggested other grounds for believing that slow-phasic fibres are immature. Since smaller fibres of the fast-phasic sort tend to have less cell substance per nucleus (cf. Table 3 and Schiefferdecker, 1928) then, on the above arguments, there may be some value in selecting animals with enlarged populations of narrow fibres.

It should also be possible to estimate the proportion of a muscle's growth potential that is expressed. This presupposes that a plot of nuclear numbers against fibre cross-sectional area takes a certain form: Consider a hypothetical situation where the number of nuclei per unit length of fibre varies from $1 \times n$ to $4 \times n$ (Fig. 5). The cross-sectional area of each nucleus is set at unity and the fibres are assumed to be

of constant sarcomere length. If all nuclei completely fail to express their growth-promoting potential, the cross-sectional area of the fibres will vary, at most, from 1 to 4 units as in the nearly vertical left hand side of the distribution in Fig. 5.

In adult animals, the cross-sectional area of fibre that represents growth sponsored by a single nucleus is much more than that of a nucleus, i.e. up to 300 times greater on average from the data of Table 3 (measured cross-sectional areas of nuclei ranged from 6 to 20 μm^2). Assume, for argument, that this area is 50 times greater in fibres

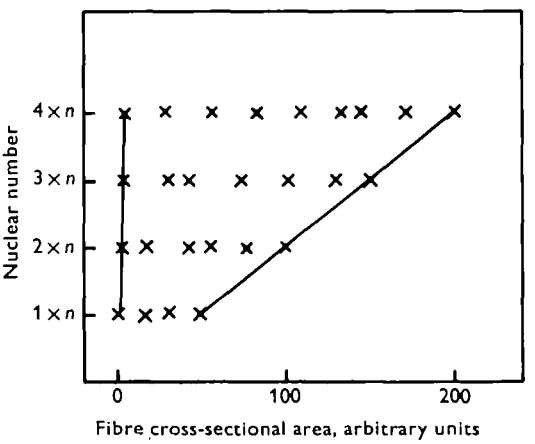

Fig. 5. Hypothetical plot of nuclear numbers against fibre cross-sectional area or (fibre breadth)^2. (For explanation, see text.)

whose nuclei are all promoting the maximum possible growth. Plots of the cross-sectional area of such fibres against nuclear number will then fall on the sloping line that represents the right hand edge of the distribution (Fig. 5). Points between the left and right hand edges will represent fibres whose growth potential has been expressed to various degrees.

The data on longitudinally oriented fibres agree quite well with this prediction The left-hand edges of the distributions are invariably fairly vertical although at higher nuclear numbers, the minimum cross-sectional areas of associated fibres are disproportionately elevated. This possibly relates to why broader fibres tend to form comparatively large amounts of cell substance per nucleus (Table 3) or it may simply reflect the fact that few fibres with particularly large numbers of nuclei were observed, thus diminishing the chances of detecting the corresponding fibres of minimally expressed growth potential.

Regarding transversely oriented fibres, lines that join fibres of minimally expressed growth are clearly more vertical than those representing the overall regression between nuclear numbers and fibre cross-sectional area. However, the former lines are less vertical than their counterparts in longitudinally oriented fibres. Again the slopes obtained from transverse sections may be influenced by the comparatively few points in the upper range of nuclear number and such points possibly represent fibres

whose complements of nuclei exceeded the maximum that was observed with reasonable frequency in longitudinally oriented fibres.

Fig. 4A, C also shows that in 2 groups of longitudinally oriented fibres, points representing the maximum growth associated with different numbers of nuclei tend to fall roughly as predicted by Fig. 5. This is less obviously so of the semitendinosus muscle of 5-month-old rats (Fig. 4B), presumably because the fibres were not expressing their maximum growth potential. Greater cross-sectional areas of fibre occur in the semitendinosus muscle of older rats (Fig. 4C).

With the possible exception of the rabbit adductor, maximum cross-sectional areas of fibre observed per nucleus in transverse sections again accord less well with the predictions of Fig. 5. For example, experimental points from the rat semitendinosus form a wedge-shaped pattern in which some broad fibres exhibit one or no nuclei and hence apparently infinite growth per nucleus. This possibly reflects sampling error associated with clumping of nuclei along the fibres. Such error should be, and appears to be reduced when nuclei are enumerated in longitudinally oriented fibre segments, i.e. in much longer lengths of fibre than is represented by a transverse section. The distribution in Fig. 3B could also reflect a certain trend for fibres with small numbers of nuclei to produce particularly large amounts of protein. However, the number that are doing so is smaller than might appear from Fig. 3 since points too closely apposed on the horizontal axis appear as one. For example, in rabbit adductor the maximum breadth[2] associated with fibres that exhibited only one nucleus was $564 \mu m^2$. In rat semitendinosus, 121 fibres displayed a single nucleus of which only 4 were broader than the above value.

The distribution of points in Fig. 4 was essentially identical when nuclear numbers per 50 sarcomeres were plotted against the volume of the corresponding fibre segments of similar length. From Fig. 4 the maximum volume of fibre produced per nucleus was found to be $24{\cdot}0 \times 10^3 \mu m^3$ for rat semitendinosus and $30{\cdot}3 \times 10^3 \mu m^3$ for rabbit adductor. From a knowledge of the corresponding sarcomere length, each experimental value in Fig. 4 was then calculated as a percentage of the maximum growth that could be sponsored by the relevant number of nuclei. Overall, rabbit adductor was found to have expressed 51·5 % of its growth potential, the corresponding figures being 39·6 and 46·3 % for rat semitendinosus at 5 and 12 months respectively. The last 2 figures were based on a value of $24{\cdot}0 \times 10^3 \mu m^3$ for maximum growth expressed per nucleus. Lower percentages will, of course be obtained for the rat muscles, if expressed relative to the value of $30{\cdot}3 \times 10^3 \mu m^3$ which was taken to represent maximally expressed growth potential in the rabbit.

Clearly, more work is required on estimating the degree to which the growth potential of muscles is expressed. Such measurements do not require the excision of entire muscles and possess an advantage over prospective estimates of total growth potential that involve knowledge of total fibre number (e.g. Stickland & Goldspink, 1975). Therefore, it may be easier to apply measurements of the sort described in this paper to the more valuable muscles of meat-producing animals.

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REFERENCES

ALLBROOK, D. B., HAN, M. F. & HELLMUTH, A. E. (1971). Population of muscle satellite cells in relation to age and mitotic activity. *Pathology* **3**, 233–243.

BURLEIGH, I. G. (1974). On the cellular regulation of growth and development in skeletal muscle. *Biol. Rev.* **49**, 267–320.

BURLEIGH, I. G. (1976). In *Meat Animals: Growth and Productivity*, NATO Advanced Study Institutes Series A 8 (ed. D. Lister, D. N. Rhodes, V. R. Fowler & M. F. Fuller), pp. 119–149. New York: Plenum.

BURLEIGH, I. G. & SCHIMKE, R. T. (1969). The activities of some enzymes concerned with energy metabolism in mammalian muscles of differing pigmentation. *Biochem. J.* **113**, 157–166.

CHEAH, K. S. (1970). The membrane-bound ascorbate oxidase system of *Halobacterium halobium*. *Biochim. biophys. Acta* **205**, 148–160.

DUTT, M. K. (1974). Specific staining of DNA aldehyde and DNA phosphate with gallamine blue. *J. Histochem.* **38**, 1–5.

GAUTHIER, G. F. (1969). On the relationship of ultrastructural and cytochemical features to colour in mammalian skeletal muscle. *Z. Zellforsch. mikrosk. Anat.* **95**, 462–482.

GAUTHIER, G. F. & PADYKULA, H. A. (1966). Cytological studies of fibre types in skeletal muscle. A comparative study of the mammalian diaphragm. *J. Cell Biol.* **28**, 333–354.

HANZLIKOVÁ, V., MACKOVÁ, E. V. & HNÍK, P. (1975). Satellite cells of the rat soleus muscle in the process of compensatory hypertrophy combined with denervation. *Cell & Tissue Res.* **160**, 411–421.

HOFFMANN, A. (1938). Der Einfluss des Trainings auf die Skeletmuskulatur. *Z. mikrosk.-anat. Forsch.* **43**, 595–622.

KOPRIWA, B. M. & MOSS, E. P. (1971). A radioautographic technique for whole mounts of muscle fibres. *J. Histochem. Cytochem.* **19**, 51–55.

LANDING, B. H., DIXON, L. G. & WELLS, T. R. (1974). Studies on isolated human skeletal muscle fibres. *Human Path.* **5**, 441–461.

LUFT, J. H. (1961). Improvements in epoxy resin embedding technique. *J. biophys. biochem. Cytol.* **9**, 409–414.

MUIR, A. R., KANJI, A. H. M. & ALLBROOK, D. (1965). The structure of the satellite cells in skeletal musculature. *J. Anat.* **99**, 435–444.

ONTELL, M. (1975). Evidence for myoblastic potential of satellite cells in denervated muscle. *Cell & Tissue Res.* **160**, 345–353.

PERRY, S. V. (1970). Biochemical adaptation during development and growth in skeletal muscle. In *The Physiology and Biochemistry of Muscle as a Food*, vol. 2 (ed. E. J. Briskey, R. G. Cassens & B. B. Marsh), pp. 537–553. Madison: University of Wisconsin Press.

PETER, J. B., BARNARD, R. J., EDGERTON, V. R., GILLESPIE, C. A. & STEMPEL, K. E. (1972). Metabolic profiles of three fibre types of skeletal muscle in guinea pigs and rabbits. *Biochemistry, Easton* **11**, 2627–2633.

RANVIER, L. (1874*a*). De quelques faits relatifs à l'histologie et à la physiologie des muscles striés. *Arch. Physiol. norm. pathol.* 2nd ser. **1**, 1–15.

RANVIER, L. (1874*b*). Note sur les vaisseaux sanguins et la circulation dans les muscles rouges. *C.r. hebd. Séanc. Acad. Sci., Paris* **26**, 28–31.

SCHIEFFERDECKER, P. (1909). *Muskeln and Muskelkerne*. Leipzig: Barth.

SCHIEFFERDECKER, P. (1928). Vergleichende Betrachtungen über 116 von mir untersuchte Muskeln. *Z. mikrosk.-anat. Forsch.* **9**, 499–539.

STICKLAND, N. G. & GOLDSPINK, G. (1975). A note on porcine skeletal muscle parameters and their possible use in progeny testing. *Anim. Prod.* **21**, 93–96.

TERÄVÄINEN, H. (1970). Satellite cells of striated muscle after compression injury so slight as not to cause degeneration of the muscle fibres. *Z. Zellforsch. mikrosk. Anat.* **103**, 320–327.

VENABLE, J. H. (1966*a*). Constant cell populations in normal, testosterone-deprived and testosterone-stimulated levator ani muscles, *Am. J. Anat.* **119**, 263–270.

VENABLE, J. H. (1966*b*). Morphology of the cells in normal, testosterone-deprived and testosterone-stimulated levator ani muscles. *Am. J. Anat.* **119**, 271–302.

WATZKA, M. (1939). Weisse und Rote Muskeln. *Z. mikrosk.-anat. Forsch.* **45**, 668–678.

YOUNG, V. R. (1970). The role of skeletal and cardiac muscle in the regulation of protein metabolism. In *Mammalian Protein Metabolism*, vol. 4 (ed. H. N. Munro), pp. 586–674. New York & London: Academic Press.

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