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THE VOLUME OF THE T-SYSTEM AND ITS ASSOCIATION WITH THE SARCOPLASMIC RETICULUM IN SLOW MUSCLE FIBRES OF THE FROG

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SUMMARY

1. A study has been made of the T-system and sarcoplasmic reticulum (SR) in slow muscle fibres of the frog, *Rana temporaria*.

2. The size of the T-system was measured by an autoradiographic method, using tritium-labelled albumin as a marker. Its volume, expressed as a fraction of that of the fibre, was found to be $1{\cdot}8 \times 10^{-3}$, as compared with a figure of $3{\cdot}9 \times 10^{-3}$ for the T-system in a twitch fibre.

3. The spatial distribution of the T-tubules, and their association with the SR, was studied with the electron microscope, employing ferritin and the enzyme peroxidase as markers. The observations show (*a*) the tubules form a *three* dimensional, rather than transverse, network and (*b*) the area of triadic (and diadic) contact with the SR is 5–10 × smaller than in a twitch fibre.

4. The possibility that the T-system and SR of the slow fibre participate in linking membrane excitation with contraction is discussed in the light of these findings.

INTRODUCTION

There are two kinds of fibre in amphibian skeletal muscles: twitch fibres, which give propagated action potentials and respond to a stimulus with a rapid contraction, and slow fibres, which contract less rapidly, respond only to repetitive stimulation and do not normally give action potentials (Tasaki & Mizutani, 1944; Küffler & Vaughan-Williams, 1953*a*, *b*; Küffler & Gerard, 1947; Burke & Ginsborg, 1956*a*, *b*; Orkand, 1963; Lannergren, 1967). It is widely accepted that the transverse tubular (T) system and the sarcoplasmic reticulum (SR) of the twitch fibre participate in the process linking membrane excitation with contraction: the T-system is thought to serve as a conducting pathway for the inward spread

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of an electrical signal which releases calcium from the SR and thus initiates a contraction (reviews: Ebashi & Endo, 1968; Sandow, 1965, 1970). Until recently, it seemed that the mechanism for regulating the contractile state of a slow fibre must differ from this, since it was reported that fibres of this type lack a T-system and have only a sparse SR (Peachey, 1961; Peachey & Huxley, 1962). However, an electron microscopic study by Page (1965), using the protein ferritin as a marker, subsequently showed that they do have a T-system, and although it appeared to be much smaller than that of the twitch fibre, it was seen to resemble the latter in two important respects: its lumen is open to the exterior, through holes in the surface membrane; and it is closely associated with the SR to form triads.

The aim of the present work was to obtain more detailed information about the size of this system of tubules and its structure. In the first part of the investigation a method similar to that described by Hill (1964) was used to measure its volume. Albumin was labelled with tritium and used as a radioactive marker. The muscles were soaked in a solution of the protein, then fixed and embedded. The quantity of radioactive material that had entered the fibres was measured by autoradiography and the fraction of the fibre volume occupied by the T-system thus calculated. Secondly, the spatial distribution of the tubules, and the extent of their association with the SR to form triads (and diads), was investigated by electron microscopy, employing ferritin and the enzyme peroxidase as markers.

METHODS

Labelling albumin with tritium

Bovine plasma albumin (Fraction V; Armour Pharmaceuticals Ltd, Eastbourne, England) was labelled with tritium by coupling it with tritiated sulphanilic acid (Roberts, 1966). The latter was prepared at the Radiochemical Centre (Amersham, Bucks., England) by a catalytic exchange method. It was purified by paper chromatography (Fewster & Hall, 1951) and assayed on a scintillation counter. Its specific activity was 1·26 Ci/m-mole.

The procedure to label a single batch of albumin was as follows. Tritiated sulphanilic acid was dissolved in water (5 mg, 1 ml.) and converted to its diazonium salt by adding hydrochloric acid (0·9 ml., 0·01 N) and sodium nitrite (0·3 ml., 100 mM). The reaction was carried out at 2° C. A solution of the albumin to be labelled (300 mg protein, 6 ml. water) was cooled to 2° C and its pH adjusted to 8 by adding sodium carbonate (200 mM). The diazotized sulphanilic acid was then slowly added and the resulting solution left for 2–3 hr at 5° C before being passed through a Sephadex column (G-50, 100 mM phosphate buffer, pH 6·9). The fraction containing the labelled albumin was collected, dialysed against running tap water (5 hr) and de-ionized water (18 hr, 5° C), and finally freeze-dried. The activity of the product ranged from 20 to 60 μCi/mg.

Experimental procedure involving the muscles

Preparation of muscles; soaking procedure. Frogs (*Rana temporaria*) were kept at 4° C until required. Most of the experiments were made with the extensor muscle of the fourth toe (ext. dig. long. IV), a few with the sartorius. The muscle to be used was dissected free, taking care not to damage its fibres, and given a preliminary soaking (30–60 min) in oxygenated Ringer solution (composition, mM (Hill, 1959): NaCl, 96; KCl, 5; $CaCl_2$, 4; sodium phosphates (pH 6·9), 5). The radioactive fluid in which it was to be soaked was prepared by dialysing a dilute solution of tritiated albumin (2–3 % (w/v), 10 ml.) against Ringer solution (3–5 l., 18 hr, 5° C), then reducing its volume approximately 10 × by negative pressure ultrafiltration through a collodion bag. The concentration of labelled protein, measured with a refractometer, was then in the required range of 20–30 %. The volume of fluid available (*ca.* 1 ml.) was sufficient to cover a muscle when it lay at the bottom of a large (1 in.) test-tube. Before the start of an experiment the tube was filled with oxygen and tightly stoppered. After 15 min the muscle was lowered into the radioactive solution and left to soak for 1–4 hr. The temperature was kept at 0° C during this time to avoid damaging the fibres by the high levels of radiation from the tritium (Hill, 1959, 1964).

At the end of the soaking period the concentration of albumin was measured with the refractometer, and a small quantity of solution removed for assay. This was weighed, diluted with de-ionized water and a sample counted in a scintillation counter. An internal standard of tritiated *n*-hexadecane (Radiochemical Centre) was used to measure the counting efficiency.

Fixing and embedding the muscles. A method of fixation was required which would retain the albumin within the muscle, and at the same time give good preservation of fine structural detail. It was shown previously (Flitney, 1966) that glutaraldehyde, a fixative which preserves the structure of a muscle well (Franzini-Armstrong & Porter, 1964; Peachey, 1965), reacts rapidly with albumin and forms a very stable product. It was also shown that loss of albumin during fixation is much less at 20° C than at 0° C. The following procedure was therefore employed. The muscle was removed from the radioactive solution and attached to a glass holder. It was gently stretched until taut, then plunged into 6 % glutaraldehyde (phosphate buffer, 100 mM, pH 6·9) where it remained for 1–2 hr at 20° C. After a brief rinse in Ringer the fixed muscle was treated with 1 % osmium tetroxide (Millonig, 1962) for a further 1–2 hr (5° C or 20° C). It was subsequently rinsed in de-ionized water, dehydrated in ethanol and embedded in Araldite (CIBA; composition: resin, 10 ml., hardener, 10 ml., accelerator (benzyldimethylamine), 0·5 ml., plasticiser (dibutyl phthalate), 0·25 ml.). Pieces of muscle were oriented in the capsules for sectioning in either transverse or longitudinal planes.

Autoradiography

Preparing autoradiographs. Sections of muscle were cut at a thickness of 1 μm. They were mounted on gelatin-coated slides, covered with Kodak AR 10 stripping film and stored either at room temperature or 5° C in light-proof boxes containing silica-gel. The exposure period varied from only a few days to several weeks, after which time the autoradiographs were developed with Kodak D 76 fine-grain developer (4 min, 20° C) and fixed in Ilford 'Hypam' fixer (1/4 dilution, 2 min, 20° C). They were then washed in running tap water (5 min), rinsed in de-ionized water and dried in air. For some purposes the finished autoradiographs were stained with basic fuchsin (Hill, 1964) to show up the pattern of the striations (Pl. 1, fig. 2). DPX was used for fixing the cover slips.

Method of analysing autoradiographs and sources of error

Identification of fibre types. The toe muscle used in most of the experiments contains both slow and twitch fibres (Gray, 1958; Peachey & Huxley, 1962). The method used to identify the two types of fibre was as follows. First, an autoradiograph of a transverse section of the muscle was photographed through the microscope at low magnification (Pl. 1, fig. 1). Thin sections (50–100 nm) were then cut from the same block and mounted on copper-mesh grids. They were lightly stained with lead citrate (Reynolds, 1963) and examined with the electron microscope. The distribution of the two kinds of fibre was recorded on the photograph, using the following structural features as the basis for their identification (cf. Page, 1965; Peachey & Huxley, 1962). Twitch fibres have relatively small myofibrils which appear roughly circular when cut transversely. They are clearly separated from one another by an investing 'sleeve' of SR. The Z-line region appears straight in cross-section, and the filaments that make up its structure are arranged in a regular square lattice pattern. At high magnification fine cross-links between the myosin filaments are seen near the centre of each sarcomere. In contrast, the myofibrils in a slow fibre are larger and only incompletely separated from each other by a sparse SR. The Z line is conspicuous and appears as a dense, amorphous region which runs a tortuous path across the fibre. There are no cross-connexions between the myosin filaments and there are relatively few mitochondria present.

Calculating the volume of the T-system from a grain count. Grain counts were made directly through a microscope fitted with a square-ruled graticule in the eye-piece. A × 80 apochromat, oil-immersion objective (N.A. 1·32) was used to view the grains. Tests with a uniformly labelled source of tritiated Araldite (see Hill (1962) for method of labelling) showed that a section of thickness 1 μm containing 1 μCi/ml. produces on average $4{\cdot}2 \times 10^{-3}$ silver grain per 100 μ^2 of emulsion per day. This figure was used to calculate the quantity of radioactive material in the fibres from the grain counts. A correction was made to allow for the fact that the activity in the embedded muscle is greater than that *in vivo* because of shrinkage during fixation and dehydration. The extent of this shrinkage was not measured, but several authors (Carlsen, Knappeis & Buchthal, 1961; Freygang, Goldstein, Hellam & Peachey, 1964; Brandt, Lopez, Reuben & Grundfest, 1967) give estimates of that incurred in their preparative schedules, and the mean of these, 37 %, was used. The volume of the T-system, expressed as a fraction of the fibre volume, is given by the activity inside the fibres divided by that of the soaking fluid.

Sources of error in the volume measurements. The error arising from the assumption of a particular value for shrinkage during processing (37 %) could be as great as 20 %, if the range for the published figures referred to above is considered. However, this will not vary appreciably because the method of preparation was always the same, and it would not in any case affect the more important comparisons which are drawn. There are three other sources of error to be considered. Two of these (the error in assaying the activity of the soaking fluid, and that which results from the assumption that the sections were all exactly 1 μm thick) are negligible in comparison with the third, which is that incurred in grain counting. The count for a population of fibres generally included 1000–3000 grains, although in some cases it was not practicable to include more than 300–500. The standard error ($\sqrt{(n/n)} \times 100$) was therefore somewhere between 3 and 6 %. If twice these values is taken to be the likely limit of the variation, it follows that the measurements are accurate to within 6–12 %.

Electron microscopy

The structure of the T-system, and its association with the SR, was studied with the electron microscope. Ferritin and peroxidase were used as markers. Bundles of fibres (twenty to thirty) from either the iliofibularis ('tonus' region) or semitendinosus muscle were used.

Experiments with peroxidase. The fibre bundles were soaked for 30 min to 2 hr in Ringer solution containing 2–10 % (w/v) horseradish peroxidase (Sigma Ltd, London). They were fixed for 15–30 min at 20° C in glutaraldehyde (composition, as above) and washed for 30 min in Ringer solution. The fixed fibres were cut transversely into short lengths (100–200 μm long) and the pieces soaked for 30–60 min at 20° C in a solution of 3,3'diaminobenzidine (5 mg; Sigma Ltd) in Tris-HCl buffer (pH 7·6, 50 mM, 10 ml.), containing 0·01 % hydrogen peroxide (Reese & Karnovsky, 1967). They were rinsed briefly in phosphate buffer (pH 6·9, 100 mM) and subsequently treated with 1 % osmium tetroxide for 12–18 hr at 20° C. The muscles were then dehydrated and finally embedded in Araldite.

Experiments with ferritin. The fibres were soaked for 1–2 hr at 0° C in Ringer solution containing 20–30 % (w/v) ferritin (ex-horse spleen; Koch-Light Ltd, Colnbrook, Bucks., England). This was prepared by dialysing 3 ml. of the commercial ferritin preparation (10 % aqueous solution) against Ringer fluid (3 × 3 l., 18 hr, 5° C), then reducing its volume to about 1 ml. by negative pressure ultrafiltration. At the end of the soaking period the fibres were fixed and embedded in the usual way.

Sectioning, staining and viewing. Sections of thickness 50–100 nm were cut and mounted on uncoated copper grids. Some were left unstained, others were lightly stained (2 min) with lead citrate. They were viewed in an A.E.I. EM 6B electron microscope.

RESULTS

The volume of the T-system

The results and experimental details for a sample of eleven muscles are summarized in Table 1. Eight of the experiments were made with the toe muscle which contains a variable proportion (generally, 10–30 %) of slow fibres. Grain counts were usually made on autoradiographs of transverse sections of muscle (Pl. 1, fig. 1), but in some instances longitudinal sections stained with basic fuchsin were used instead; the slow type of fibre is easily recognized in such material because it has longer sarcomeres (Page, 1965) and a less regular striation pattern (Pl. 1, fig. 2). The counts included all the slow fibres in a muscle, since there were so few present, and a sample of its twitch fibres (ten to twenty). However, in three of the muscles studied (32, 40A and 41B) all of the fibres were represented in the analyses.

The size of the space accessible to the albumin, expressed as a percentage of the fibre volume, is given in columns 5 and 6. The results show that the compartment into which the albumin diffuses in the slow fibre (mean volume ± S.D.: 0·18 ± 0·02 %; sixty fibres; seven muscles) is about half as large as that in the twitch fibre (mean volume ± S.D.: 0·39 ± 0·03 %; 291 fibres; eleven muscles). The comparison is more meaningful if the average value for the slow fibres in a muscle is given as a fraction of that

TABLE 1. The volume of the T-system in twitch and slow fibres

Expt.	Muscle	Radioactivity of solution (μCi/ml.)	Period in soaking solution (hr)	Volume of T-system (% fibre volume) in Twitch fibres	Volume of T-system (% fibre volume) in Slow fibres	Ratio slow/twitch
22	Sartorius	1893	1·0	0·45 (44)	—	—
25	Sartorius	5430	1·2	0·49 (28)	—	—
40B	Sartorius	3945	2·0	0·41 (15)	—	—
32	Toe	1150	1·0	0·42 (45)	0·18 (13)	0·43
40A	Toe	3950	2·0	0·27 (49)	0·14 (10)	0·52
41B	Toe	4050	2·5	0·21 (39)	9·11 (7)	0·52
74	Toe	4760	2·0	0·35 (18)	0·24 (8)	0·69
73A*	Toe	3630	1·2	0·27 (10)	0·17 (9)	0·63
73B*	Toe	3630	1·2	0·28 (16)	0·20 (6)	0·71
76C	Toe	13,210	1·3	0·41 (17)	0·22 (7)	0·54
67	Toe	2700	1·0	0·38 (10)	—	—
	Mean ± S.D. mean for all muscles:			0·39 ± 0·03	0·18 ± 0·02	0·58
	Coefficient of variation (100 × S.D./mean):			8%	11%	—
	Total number of fibres:			291	60	—

* Results for a pair of muscles. The figures in brackets give the number of fibres included in each grain count.

for its twitch fibres, since this eliminates differences due to variation between animals. The figures for seven of the muscles studied, given in column 7, range from 0·43 to 0·71, with a mean of 0·58.

The assumption is that these 'albumin spaces' are equivalent to the T-system, which implies that there is no entry of radioactive material into any other part of the fibre. There can be little doubt that this is so. It is improbable that a molecule as large as that of albumin could leak into the fibres through their surfaces; or that any highly active labelled contaminant was present which could have done so, because the soaking fluid was

TABLE 2. The space accessible to albumin in paired muscles exposed to the radioactive fluid for different times

Expt.	Muscles	Soaking period (hr)	Volume of 'albumin space'* in Twitch fibres	Volume of 'albumin space'* in Slow fibres
A	Sartorii	0·5	0·30 (15)	—
		4·0	0·33 (17)	—
B†	Sartorii	2·0	0·68 (20)	—
		4·0	0·73 (19)	—
C	Toe	1·0	0·38 (20)	0·14 (8)
		3·0	0·40 (21)	0·16 (6)
D	Toe	0·5	0·46 (13)	0·21 (9)
		4·0	0·42 (14)	0·25 (5)

* Grain counts were restricted to surface fibres only, to ensure that the analysis included only those fibres which had attained equilibrium with the external solution.

† The values for this pair of muscle were unusually high. The figures in brackets give the number of fibres included in each grain count.

carefully purified before the start of an experiment. Both of these assumptions are borne out by the results of some experiments with paired muscles (i.e. muscles taken from right and left limbs of the same animal) which were exposed to the radioactive solution for different times. The soaking period for one muscle of each pair greatly exceeded the minimum time required for albumin to enter the T-system (estimated to be 10–12 min; see below), while that for its partner was much shorter, though still adequate for its fibres to approach diffusion equilibrium with the external fluid. These experiments gave no evidence for a time-dependent increase in the amount of radioactive material entering the fibres (Table 2), such differences as were observed being within the limits of the experimental error.

Hill (1964) estimates that the time required for the T-system to approach diffusion equilibration with the external fluid (> 90 % saturation, fibre diameter, 70 μm) is about 100 min. It was assumed in calculating this figure that the diffusion constant for albumin in the narrow T-tubules is 100 × smaller than in free solution, but for

reasons given in his paper (p. 277), Hill concludes that the restriction factor is probably very much less than this. The above estimate of 10–12 min (> 93 % saturation, fibre diameter, 100 μm) assumes a figure of only 10. The shortest soaking period actually given was 30 min, and the autoradiographic analyses were restricted to outer fibres only.

There are other grounds for concluding that the T-system is the only region accessible to albumin in appreciable amounts. Observations with the electron microscope show that both ferritin and peroxidase are excluded from the rest of the fibre, including the SR. However, numerous vesicles are to be found in the vicinity of the fibre membrane, and this raises the question of the possible entry of albumin by micropinocytosis. The results in Table 2 show that if albumin is taken up by this means, the amount involved must be very small by comparison with that which diffuses into the T-system.

The structure of the T-system and its association with the sarcoplasmic reticulum

The results so far presented provide evidence that the T-system in the slow fibre is much larger than has hitherto been supposed, and in view of this unexpected finding it was decided to make a study of its structure. Particular attention was given to the extent of its association with the SR, since it is this feature which is of physiological interest.

The observations that follow were made on muscles previously soaked in solutions containing ferritin or peroxidase.

The T-system. In a twitch fibre the T-system is made up of transversely aligned tubules which encircle the myofibrils at about the level of each Z line (Huxley, 1964; Page, 1964, 1965; Peachey, 1965; Peachey & Schild, 1968). There are occasional longitudinal tubules which connect adjacent networks of neighboring sarcomeres, but these are rare and the system is predominantly two-dimensional (or transverse) in its arrangement. The spatial distribution of the T-tubules is strikingly different from this in the slow fibre. Here the T-system takes the form of a loosely fitting, *three*-dimensional network (Pl. 4) in which longitudinal tubules account for a large proportion, estimated to be as much as 50 %, of its volume. The shape of the tubules also differs from those of the twitch fibre; when cut transversely they appear roughly circular (rather than elliptical) in outline, with diameters ranging from 28 to 33 nm.

The SR. The slow fibre is only incompletely divided into myofibrils, giving rise to the typical 'felderstruktur' appearance when viewed with the light microscope (Kruger, 1952; Peachey & Huxley, 1962; Gray, 1958) and its SR is less extensive than that of the twitch fibre. It also differs in its organization (Pl. 2). There are no large cisternae present to interrupt

the longitudinal continuity of the system, and consequently many of its tubules (diameters 50–70 nm) pass from one sarcomere to the next. There is no precise counterpart of the fenestrated collar, a prominent feature of the SR in a twitch fibre (Peachey, 1965), although longitudinally oriented tubules converge near the centre of the sarcomere and there is often a few perforations through this part of the reticulum. The so-called intermediate cisternae are also lacking, and there are no grossly expanded regions to compare in size with the terminal cisternae of the triads in a twitch fibre.

The area of triadic/diadic contact between the T-system and SR. The T-system and SR form triads which resemble those seen in a twitch fibre, as well as two-component structures termed diads (Page, 1965). The gap separating the confronting membranes of a triad or diad (15–17 nm wide) lacks the structural complexity which is characteristic of this zone in a twitch fibre (Kelly, 1969), although the electron opacity of the apposed membranes is greater than in those regions where there is no association between the two (Pl. 3, figs. 1, 2).

The part of the reticulum associated with a T-tubule is generally swollen to form a bulbous, sometimes elongated, sac, but this does not form a continuous 'collar' around the fibril and, accordingly, only a relatively small proportion of the available area of T-system membrane can form triads or diads. It is estimated, from a sample of thirty-five micrographs similar to those in Pl. 3, figs. 1, 2 that this probably amounts to no more than 15–30% of that potentially able to make such contact; the rest of the membrane is 'bare'. On the other hand, it is reported (Peachey, 1965) that as much as 80% of the T-system wall is utilized for this purpose in the twitch fibre.

It may be shown that these figures actually represent a five-to tenfold difference in the amount of triadic/diadic contact as between the two kinds of fibre. The magnitude of this difference becomes apparent only when the size and shape of the T-tubules is taken into account, as well as the fraction of the fibre volume that they occupy (see below). Table 3 shows that, with the exception of the obvious difference in the spatial distribution of the T-tubules, already referred to, the extent of the contact between the T-system and SR is the only feature which differs greatly in the two kinds of fibre.

In calculating the total area of membrane forming triads and diads the volume of the T-system in the slow fibre is taken as $1{\cdot}8 \times 10^{-3}$ and that of the twitch fibre as $3{\cdot}6 \times 10^{-3}$ (Table 1). It is assumed that the T-tubules are cylindrical and that their average diameter is 30 nm (cf. Page, 1965). Those of the twitch fibre are assumed to be elliptical, the dimensions of the major and minor axes being 110 nm and 25 nm respectively (Page, quoted by Hill, 1964). The usual formulae are used for the volume and surface area of a cylindrical tubule; the surface area of an elliptical tubule is $2\pi l\sqrt{\frac{1}{2}(a^2+b^2)}$ and its volume $2\pi abl$, where a and b are the two semi-axes, and l is

the length. The proportion of T-tubular wall involved in forming triads and diads is taken as 15–30 % for the slow fibre, and 80 % for the twitch fibre.

The values obtained for several parameters of the two T-systems are listed in Table 3.

TABLE 3. Parameters of twitch and slow fibre T-systems

Parameter	Units	Slow fibre	Twitch fibre
Volume of T-system*	cm^3/cm^3 fibre†	$1{\cdot}8 \times 10^{-3}$	$3{\cdot}6 \times 10^{-3}$
Volume of tubules	cm^3/cm^2 fibre surface	$2{\cdot}3 \times 10^{-6}$	$4{\cdot}5 \times 10^{-6}$
Surface area of tubules	cm^2/cm^2 fibre surface	3·0	5·3
Surface area of tubules	cm^2/cm^3 fibre	$2{\cdot}4 \times 10^3$	$4{\cdot}2 \times 10^3$
Length of tubules	cm/cm^3 fibre	$2{\cdot}5 \times 10^8$	$1{\cdot}7 \times 10^8$
Area of T-tubule membrane involved in forming triads/diads	cm^2/cm^2 fibre surface	0·5–0·9	4·2
Surface area T-tubules/unit volume of T-tubule	cm^2/cm^3 T-tubule	$13{\cdot}3 \times 10^5$	$11{\cdot}8 \times 10^5$

* Table 1.

† The results are all calculated for a fibre diameter of 50 μm.

DISCUSSION

It is generally thought that the slow fibre lacks a proper T-system (reviews: Hess, 1967, 1970), but the evidence presented here does not support this view; indeed, it seems clear from what has been said that an elaborate T-system network is present in such fibres. What is the reason for this 'discrepancy'? It is known that the appearance of the T-system and SR is critically affected by the type of fixative which is used: osmium tetroxide by itself does not give good preservation of these components, whereas fixation with glutaraldehyde generally gives excellent fine-structural detail (Franzini-Armstrong & Porter, 1964; Peachey, 1965). The earlier electron microscopic studies of the slow fibre were made with osmium tetroxide alone, and this is presumably the reason why T-tubules were never observed. It has been shown by Page (1965), and confirmed here, that glutaraldehyde preserves their structure well, and they are readily seen if a marker such as ferritin or peroxidase is employed.

There is a great deal of evidence to show that the T-system serves to conduct an electrical signal into the interior of the twitch fibre, and that this causes calcium to be released from the SR (Ebashi & Endo, 1968). The existence of a comparable system of tubules, and its close association with the SR to form triadic structures, strongly suggests that a similar process links membrane activity with contraction in the slow fibre also.

If this is the case, then a question which should be asked is: why is the area of contact between the T-system and SR so much smaller than in a twitch fibre? The explanation is probably to be found in a comparison of

the maximal rates at which the sliding filaments can interact. Measurement of the heat production during a tetanus (Floyd & Smith, 1971) and of the shortening velocity of the sarcomeres in 'skinned' fibres (Costantin, Podolsky & Tice, 1967) and intact fibres (Peachey, quoted by Costantin *et al.* 1967) show that this is probably 10–30 × less in the slow fibre. The myofibrillar ATP-ase activity, the property which ultimately determines contractile performance (Barany, 1966), has also been shown, by histochemical means (Engel & Irwin, 1967), to be low in the slow fibre. It is therefore reasonable to suppose that since its myosin is capable only of a comparatively slow rate of activity, the rate at which it must be supplied with calcium need not be so great as in a twitch fibre. Since the rate at which this calcium is released and made available for contraction presumably depends upon the area of contact between the T-system and SR (cf. Page, 1968), it is not surprising to find that this is relatively small in the slow fibre.

Stefani & Steinbach (1968) have reported that a form of treatment with glycerol which severely disrupts the T-system in a twitch fibre and 'uncouples' membrane activity from contraction (Howell, 1969) has no effect on the excitability and contractility of the slow fibre, and this led them to doubt that its T-system could be involved in initiating contraction. However, they offered no evidence to show that the slow fibres in their preparations actually suffered any structural damage; even in a twitch fibre the T-tubules sometimes escape destruction (Howell, 1969; see also below), and it is possible that those of the slow fibre are relatively more resistant to such treatment.

A few experiments were made in an attempt to get some evidence on this point. It was found that subjecting a muscle to Howell's glycerol treatment before soaking it in the radioactive solution did not prevent albumin from entering its fibres. The size of the space accessible to albumin in the slow fibre was not significantly affected by the treatment and, surprisingly, as much as 50 % (average, four muscles; range 33–72 %) of the original albumin space in the twitch fibre also remained accessible to the protein. This confirms what has been said above about the difficulty of disrupting the T-system; the damage sustained by the twitch fibres was very much less than had been anticipated (cf. Eisenberg & Eisenberg, 1968; Nakajima, Nakajima & Peachey, 1969; Krolenko, 1969). The results are also consistent with the suggestion that the T-tubules in a slow fibre are even more resistant to glycerol than are those of a twitch fibre.

Local activation experiments with the slow fibre have shown that both A and I bands of its sarcomeres are responsive to stimulation and that the resulting contraction spreads out in all directions from the depolarized area of membrane (Peachey & Huxley, 1960). This behaviour is very different from that of a twitch fibre where local stimulation of I (but not A) bands elicits a contraction which spreads in the transverse plane only (Huxley,

1959). It is consistent with the finding that T-tubules in a slow fibre open to the surface in both A and I bands (Page, 1965), and with the fact demonstrated here that they form a three-dimensional pattern, rather than a transverse one.

Costantin *et al.* (1967), using 'skinned' preparations, showed that the SR in a slow fibre is capable of accumulating calcium, although it appears to be less effective in doing so than that of a twitch fibre. It is known that the contractile properties of the slow fibre are more dependent on the concentration of calcium in the external solution than are those of the twitch fibre (Peachey, 1961; Schaectelin, 1961; Pauschinger & Brecht, 1961; Nasledov, Zachar & Zacharova, 1966; Lannergren, 1967), and if activation is initiated via the T-system, this would appear to indicate that the calcium ready to be released from the triadic region is stored in a form which is more loosely bound and hence more readily exchangeable than that in a twitch fibre.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. An autoradiograph of a transverse section (unstained) through a toe muscle containing tritiated albumin. The individual silver grains that make up the autoradiographic image are visible only at much higher magnification (Pl. 1, fig. 2). Those lying over the heavily labelled inter-fibre spaces are conspicuous and appear as a network of black lines delineating the fibres. Photographs such as this were used when mapping the distribution of the two kinds of fibre, as described in the text. Autoradiograph 116. Exposure period, 21 days. Scale bar, 100 μm.

Fig. 2. An autoradiograph of a longitudinal section of muscle stained with basic fuchsin to show up the pattern of the striations. The A bands and Z lines take up the stain, the I bands do not. Two fibres are shown, separated by a heavily labelled inter-fibre space. The upper one is a twitch fibre, and the lower one, with longer sarcomeres, is a slow fibre. Autoradiograph 54. Exposure period, 40 days. Scale bar, 10 μm.

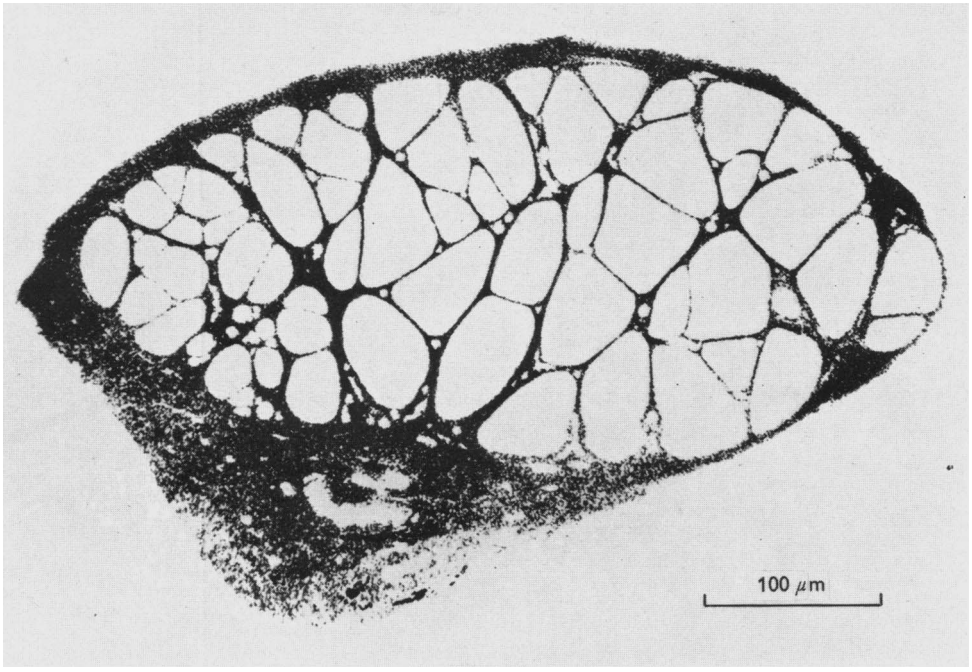


Fig. 1

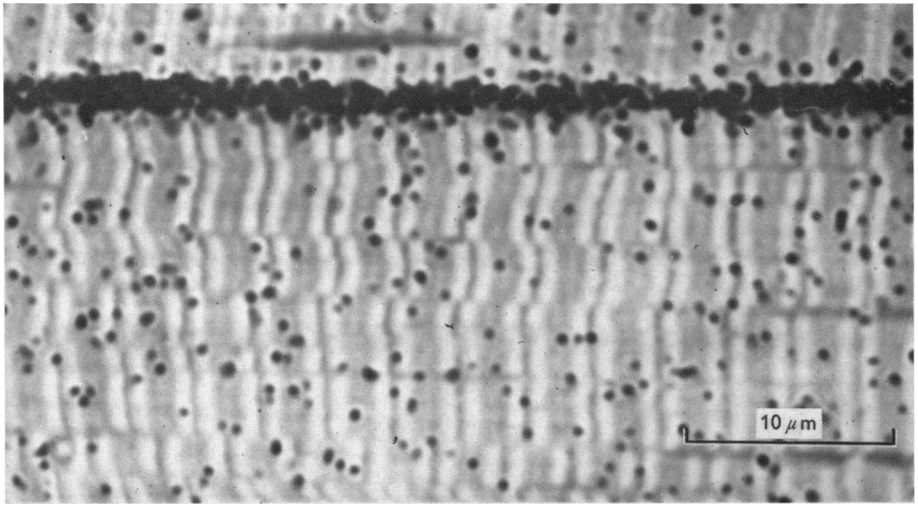
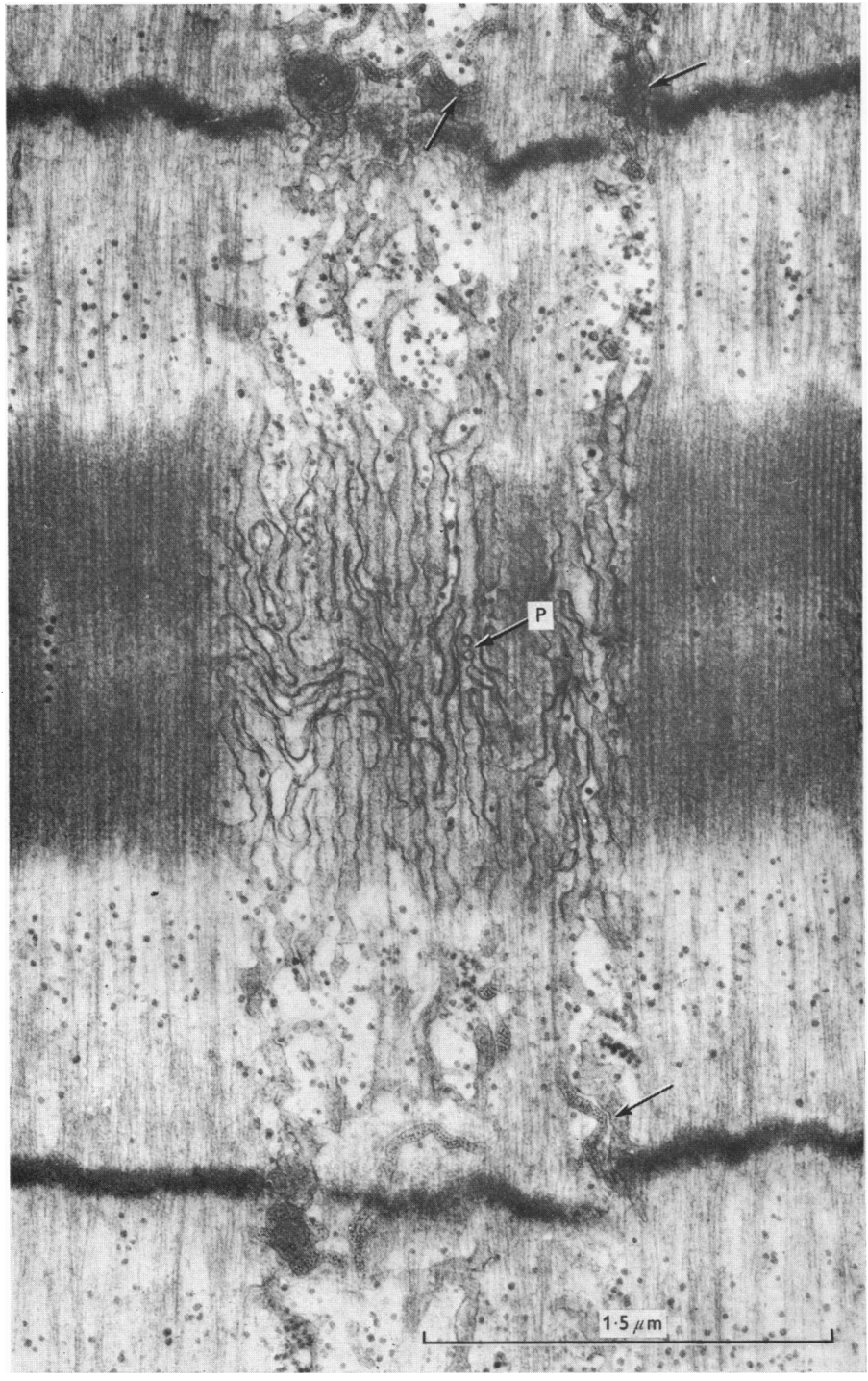


Fig. 2



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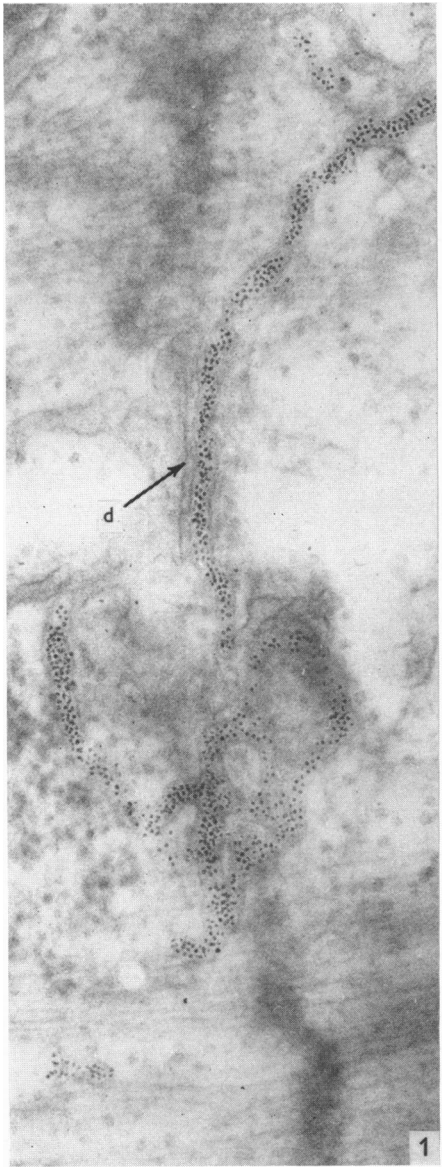

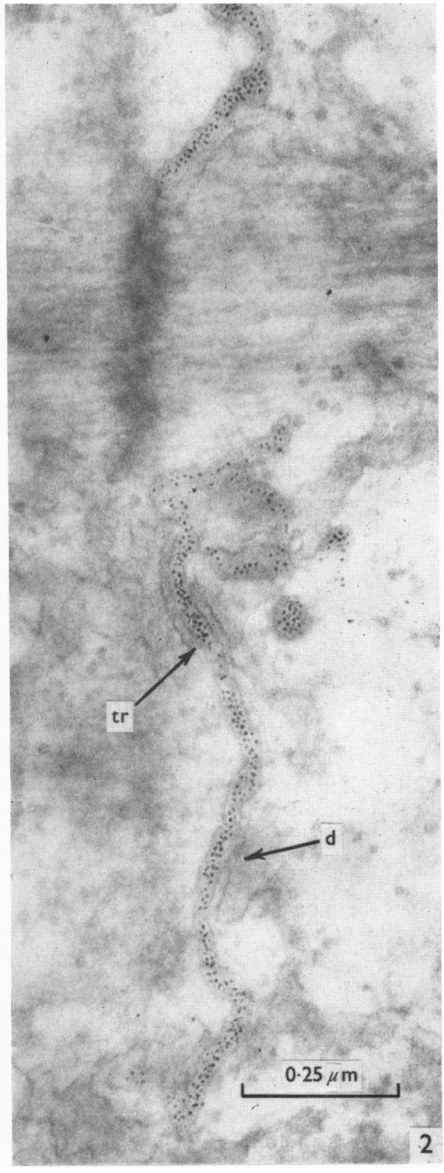

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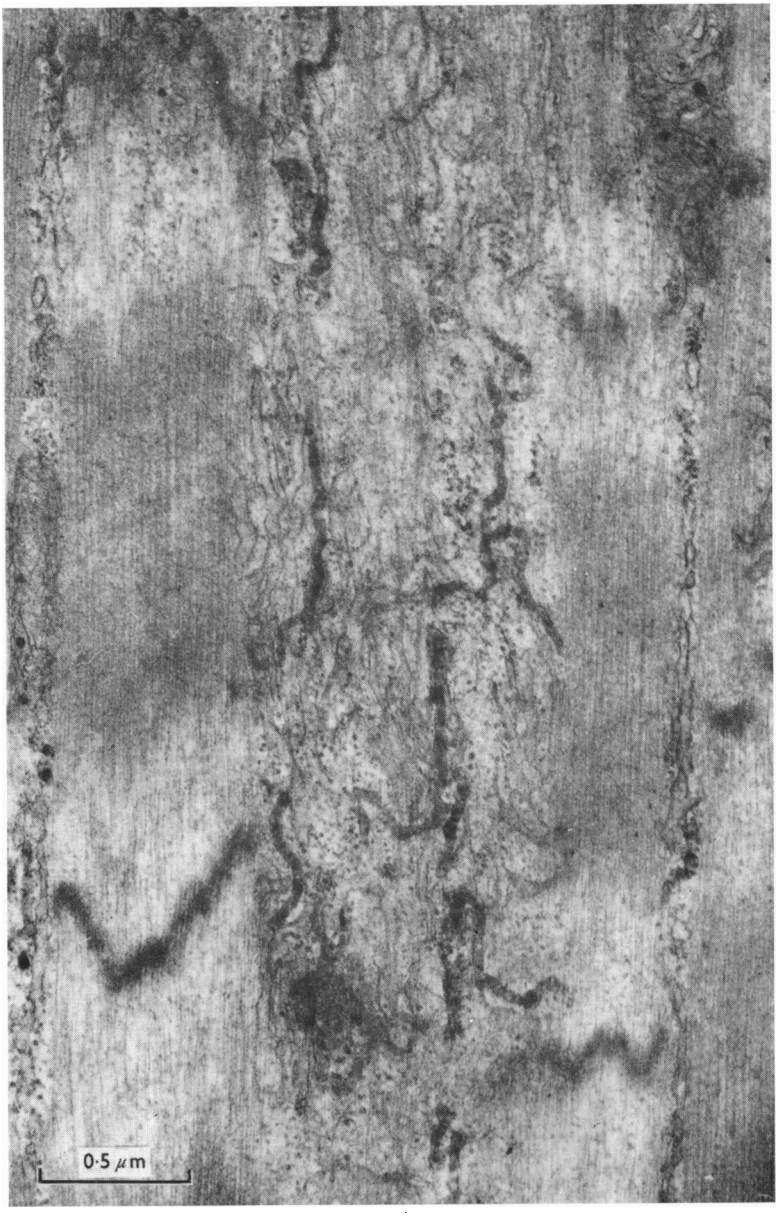

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PLATE 2

The organization of the SR is illustrated in this micrograph of a slow fibre. The T-tubules containing ferritin are seen at each end of the sarcomere where they are associated with elements of the SR to form regions of triadic/diadic contact (arrows). The longitudinal tubules of the reticulum converge near the centre of the sarcomere; there are a few perforations (P) through this region which are reminiscent of the holes in the fenestrated collar of the SR in a twitch fibre. Section stained with lead citrate. Scale bar. 1·5 μm.

PLATE 3

Figs. 1 and 2. Longitudinal sections through a slow fibre showing regions of triadic (tr) and diadic (d) contact between the T-tubules (containing molecules of ferritin) and enlarged elements of the SR. These two micrographs show successive Z-line regions of the same myofibril. Note that only a fraction of the area of T-tubule wall shown is associated with the SR to form triads/diads. Sections unstained. Scale bar, 0·25 μm.

PLATE 4

A longitudinal section through a slow fibre containing peroxidase. The presence of the enzyme in the T-tubules is indicated by the dense reaction product filling their lumen. The three-dimensional nature of the T-system network is apparent, with both longitudinal and transverse tubules represented in the same region. Note that the T-tubules appear to fit less tightly round the myofibrils than do those of the twitch fibre. Section stained with lead citrate. Scale bar, 0·5 μm.