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FLEXION AND EXTENSION

Studies employing muscles of the shank in amphibians.

A Thesis submitted for the degree of Doctor of Philosophy

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SUMMARY

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Tibialis anticus and peronaeus longus of the frog have been examined by optical and electron microscopy. Sodium and potassium concentrations of these muscles have been determined. Double myograms have been used to compare physiological and pharmacological properties of flexor and extensor and to demonstrate various patterns of convulsion and their modification by depressants. Contractures have been investigated in the isolated organ bath. Depolarizing agents have been used to compare the proportions of slow fibres in the gastrocnemius of frogs and toads.

Tibialis anticus, a flexor, has been shown to be heterogenous, consisting of twitch and slow fibres, and peronaeus longus to be homogenous, containing only twitch fibres. The twitch fibres of the flexor are shown to be fast compared with the twitch fibres of the extensor and their resting potentials, as deduced from potassium concentrations, are found to differ. Evidence is collated to evince the principle that a flexor is heterogenous to effect the functions of rapid withdrawal and of maintaining that withdrawal, whereas an extensor is homogenous since it merely exerts a thrust. Analogies between amphibian and mammalian muscle fibres are discussed.

Experiments with centrally-acting drugs have revealed differences of action on the control of flexors and extensors. The complex patterns of convulsion render quantitative determination of the activities of flexor and extensor difficult, so a full assessment of the significance of the results must await the development of more precise techniques. The duration of the longest-sustained convulsion has been used as a basis for comparison.

The gastrocnemius of toads was found to contain a higher proportion of slow fibres than that of frogs and it is suggested that this is an adaptation to walking as compared with leaping.

CONTENTS

INTRODUCTION

Functional adaptation is a basic principle of biology, pervading the whole field of that science. Adaptation is in part inherited and in part acquired. Natural selection was responsible, for example, for the adaptation of the teeth and legs of the ancestors of the horse to the lengthening grass of their habitat, whereas the development of the biceps muscles of the pugilist is acquired in the gymnasium and the ring. Since both evolution and individual usage produce gradual changes, intermediate forms may be expected to persist or to reappear and to constitute difficulties for the physiologist, just as they do in taxonomy. An important factor stressed by Barcroft (1934) is that every adaptation is an integration.

In neuromuscular physiology, the adaptations to the functions of flexion and extension have not been fully elucidated. The main contribution has been made by Sherrington (1906) but was confined to reflexes. The activity of the flexor is directed to the rapid removal of a limb from a source of injury and maintaining that withdrawal, whereas the extensor exerts a thrust. The adaptation is likely to involve the muscle fibres, the nerve-endings, the nerve fibres, and the central nervous system.

In the shank of an amphibian, there are three main muscles: tibialis anticus, which is a flexor (dorsiflexor), invertor, and adductor of the ankle; peronaeus longus, which is an extensor (plantar flexor), evertor, and abductor of the ankle, and gastrocnemius, which is a flexor of the knee and an extensor of the ankle. In the forearm, the adoption of a plantigrade posture involves a rotation, whereby a muscle which is phylogenetically a flexor may become an extensor, and vice versa; moreover, clasping is an activity more akin to the

extensor thrust than to the defensive flexor reflex.

The original practical work which has been carried out in the present project includes an examination of the muscles by optical and electron microscopy, by flame photometry, and by electrophysiological techniques, a pharmacological investigation of the neuromuscular junction, and a study of the central actions of convulsants and their antagonists. A comparative study of various species of frogs and toads has only been extended to the pharmacology of the neuromuscular junction.

The confusion between fast/slow (white/red) muscle fibres of mammals and fast/slow (twitch/tonic) fibre systems of amphibians adds difficulty to the application of comparative physiology in this field. An attempt has been made to clarify the position.

THE HISTOLOGICAL STRUCTURE OF THE MUSCLES

A. OPTICAL MICROSCOPY

Kruger (1952) linked with tonic activity muscles whose fibres show Felderstruktur, served by finely myelinated nerve fibres bearing diffuse endings 'en grappe', and with tetanic activity muscle fibres of Fibrellenstruktur, served by thickly myelinated nerve fibres terminating 'en plaque'. His monograph does not include a description of the structure either of tibialis anticus, or of peronaeus longus in Amphibia. Sommerkamp (1928) classified the muscles of the frog into (a) those which are purely tetanic; (b) those containing slow and twitch fibres randomly distributed, and (c) those containing tonus bundles and a purely tetanic portion. He did not find a muscle of the second type (b) in the hind limb, but, of those attached to the shoulder girdle, most were flexors, but there were also some extensors.

Gastrocnemius, in the frog, is mainly a purely tetanic muscle, but it contains two tonus bundles. The tetanic part is composed exclusively of fibres with Fibrellenstruktur, whereas the tonus bundles contain fibres with both Fibrellen- and Felder-struktur (Kruger, 1952). The nature of tibialis anticus and of peronaeus longus required identification.

METHOD: Routine histological preparations were made from the upper, middle, and lower regions of tibialis anticus and from the middle of peronaeus longus (Fig. 1). The sections were taken from muscles allowed to shorten whilst being fixed in Bouin's fluid prior to embedding in paraffin wax and were stained with haematoxylin and eosin. Five slides, each bearing three to five serial sections, were prepared from each region.

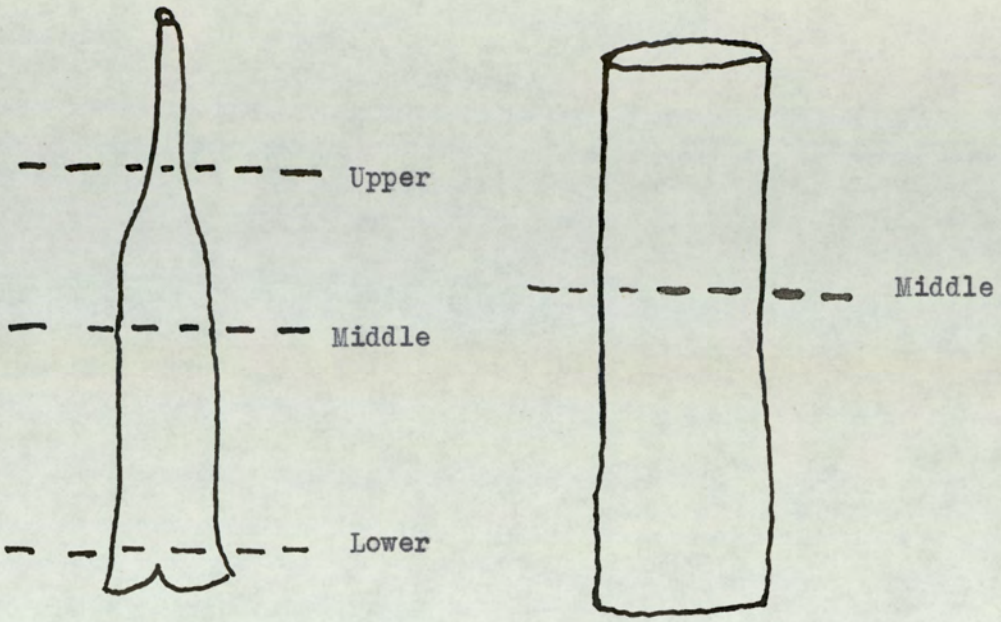


Fig. 1: Location of sections taken from muscles of the frog (Rana temporaria).

RESULTS: Plates 1 - 3 represent sections through the three regions of tibialis anticus at a magnification of x 65. From these, it is clear that this muscle does not contain a tonus bundle, or bundles. Plate 4 is a section through peronaeus longus at the same magnification.

Plate 5, at a magnification of x 410, illustrates the random distribution of slow and twitch fibres in the middle portion of tibialis anticus. A similar distribution was observed in the upper and lower parts of this muscle.

Plates 6 and 7, at a magnification of x 1,040, are through the middle portions of tibialis anticus and of peronaeus longus, respectively. Tibialis anticus possesses slow and twitch fibres, whereas peronaeus longus consists exclusively of twitch fibres. The twitch fibres of tibialis anticus are smaller in diameter than the twitch fibres of peronaeus longus. The slow fibres of tibialis anticus do not differ

appreciably, in diameter, from the twitch fibres in this muscle.

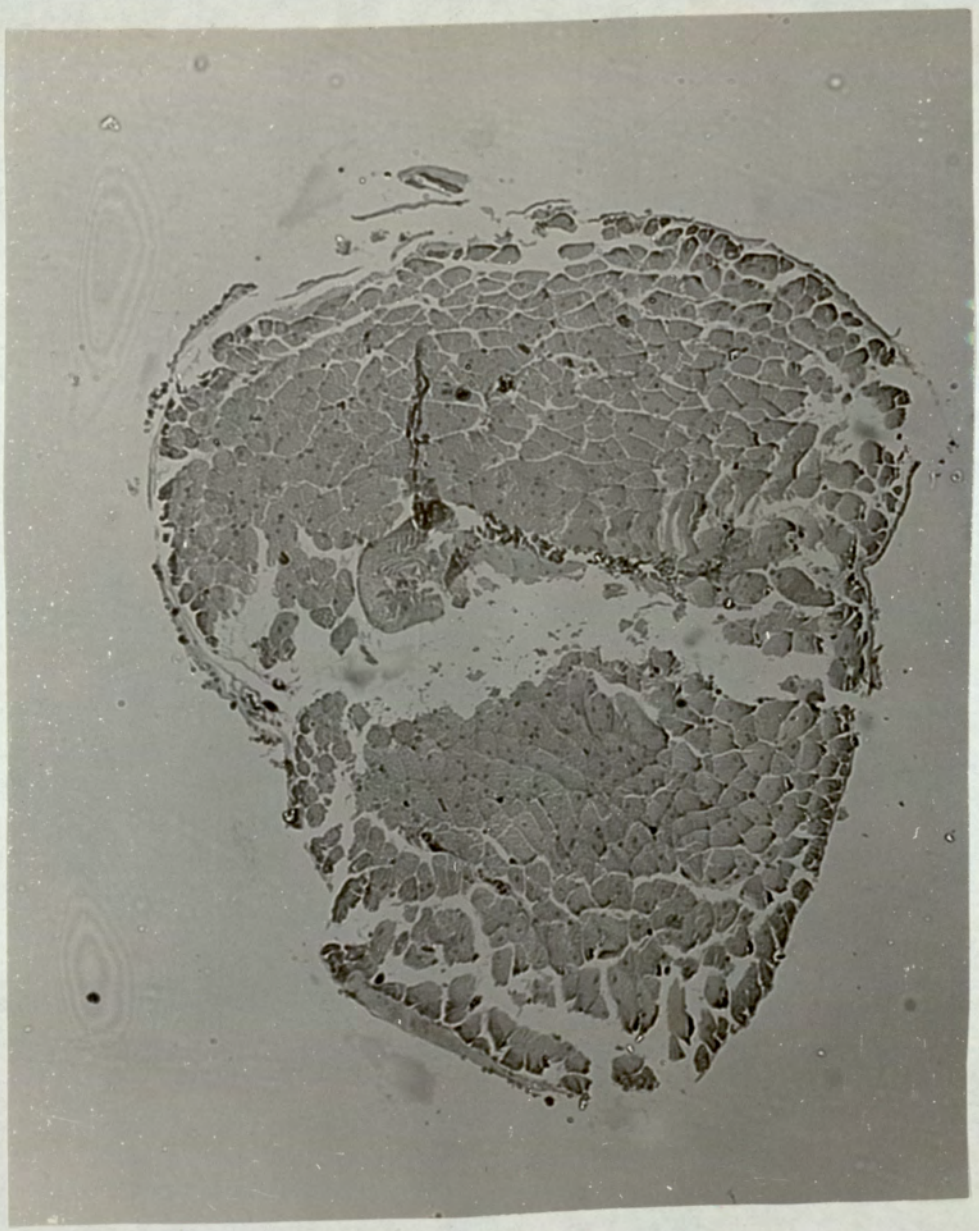


Plate 1: Tibialis anticus of frog (Rana temporaria). T.S. through upper portion, x 65.

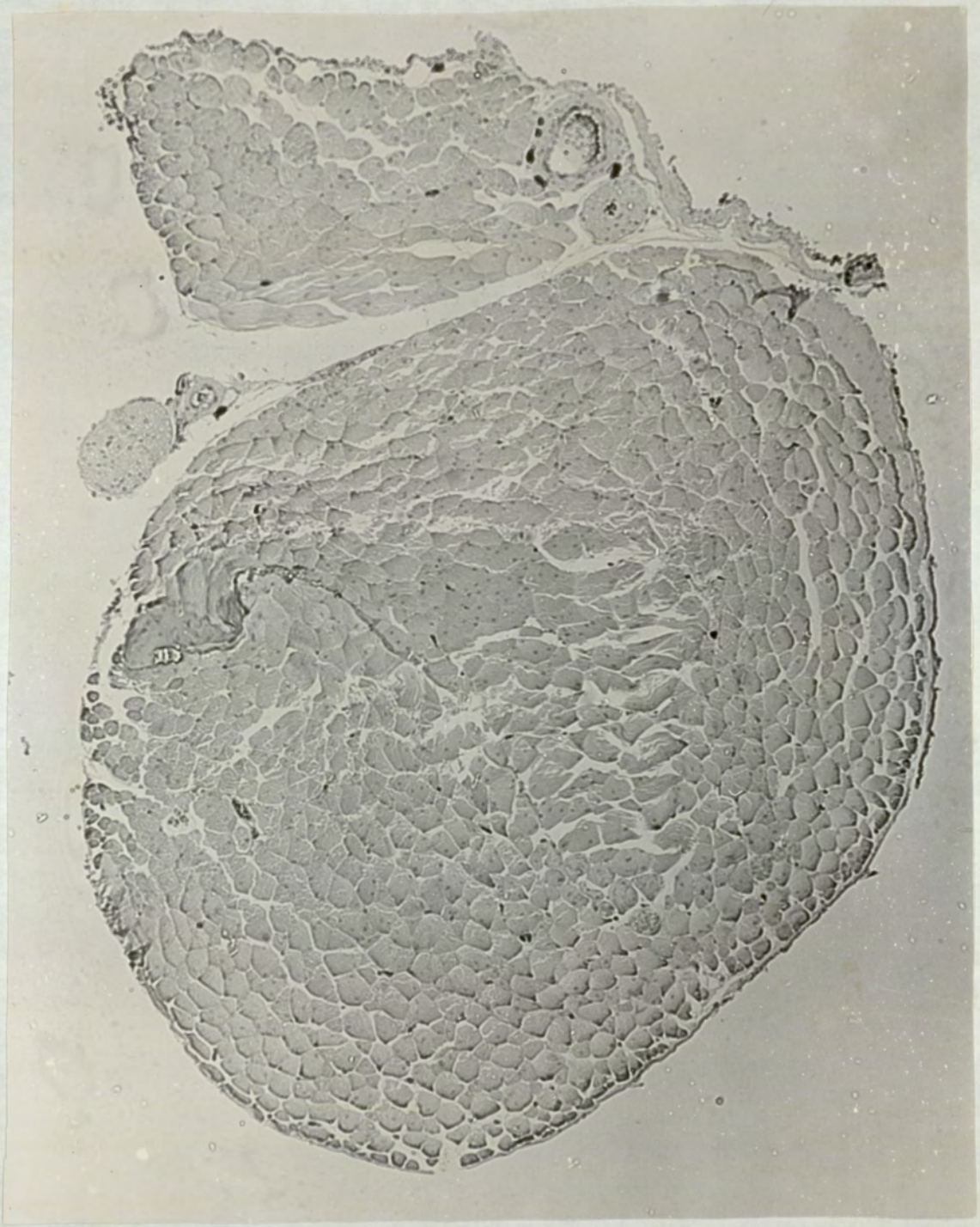


Plate 2: Tibialis anticus of frog (Rana temporaria). T.S. through middle portion, x 65.

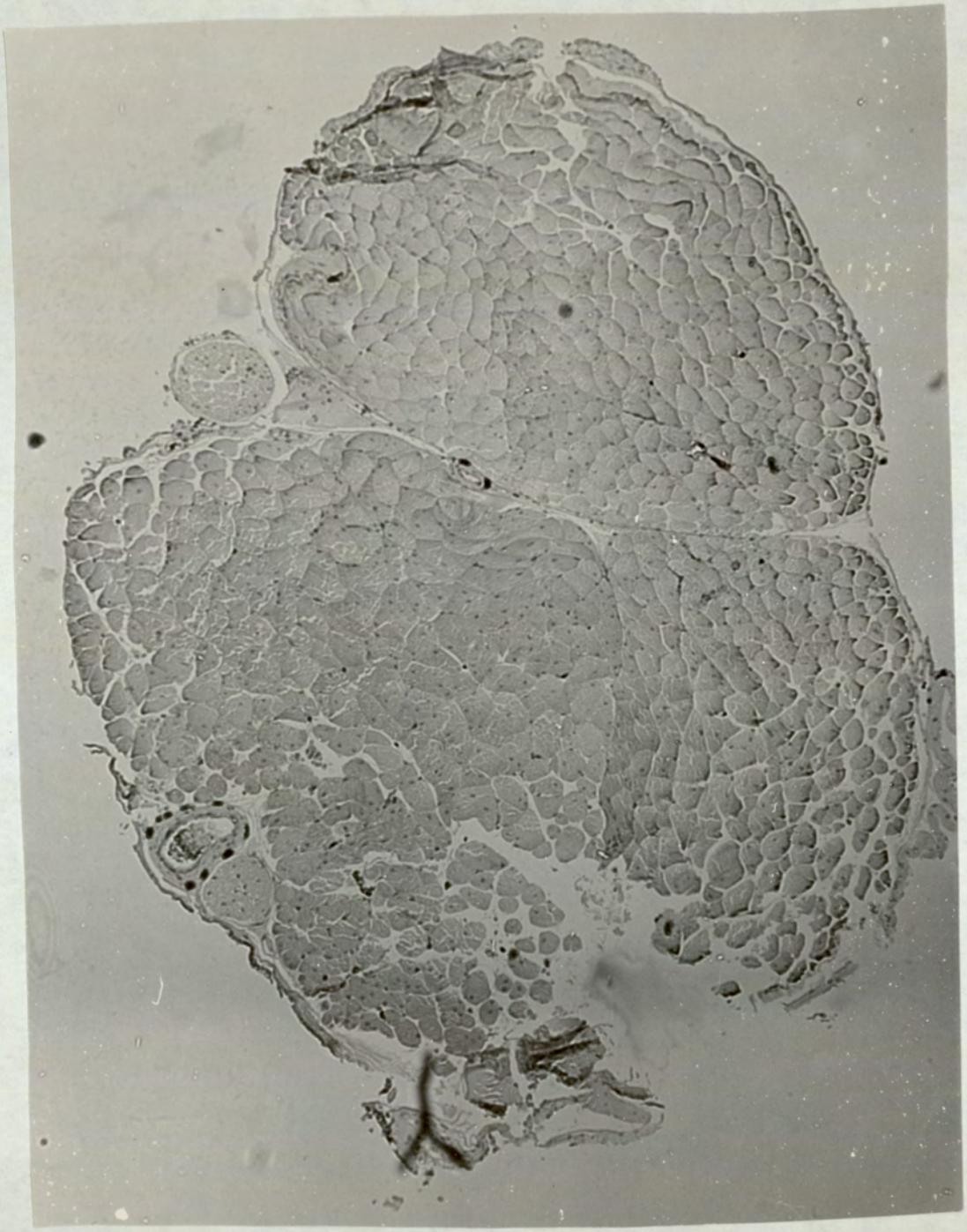


Plate 3: Tibialis anticus of frog (Rana temporaria). T.S. through lower portion, x 65.

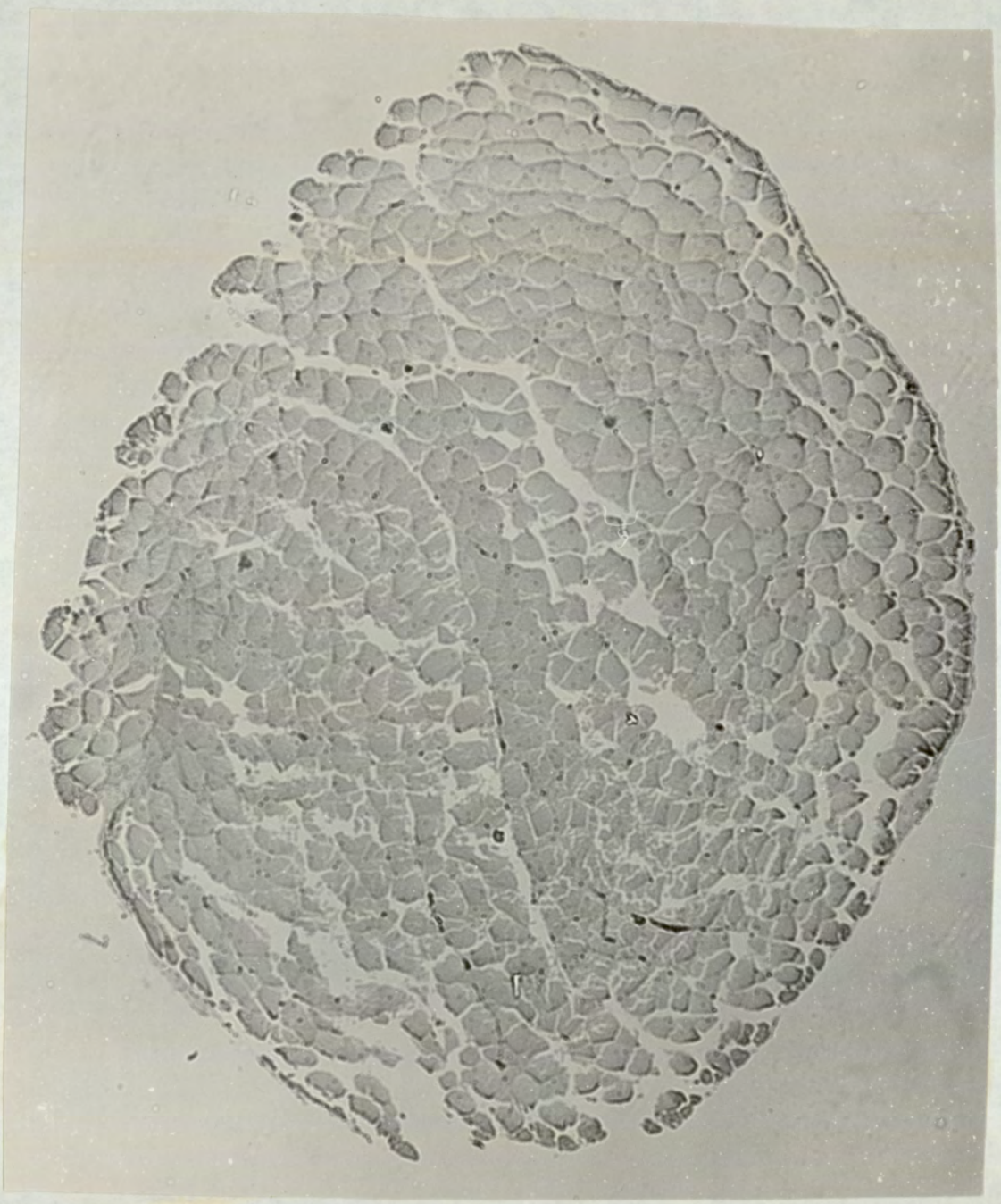


Plate 4: Peronaeus longus of frog (Rana temporaria). T.S. through middle portion, x 65.

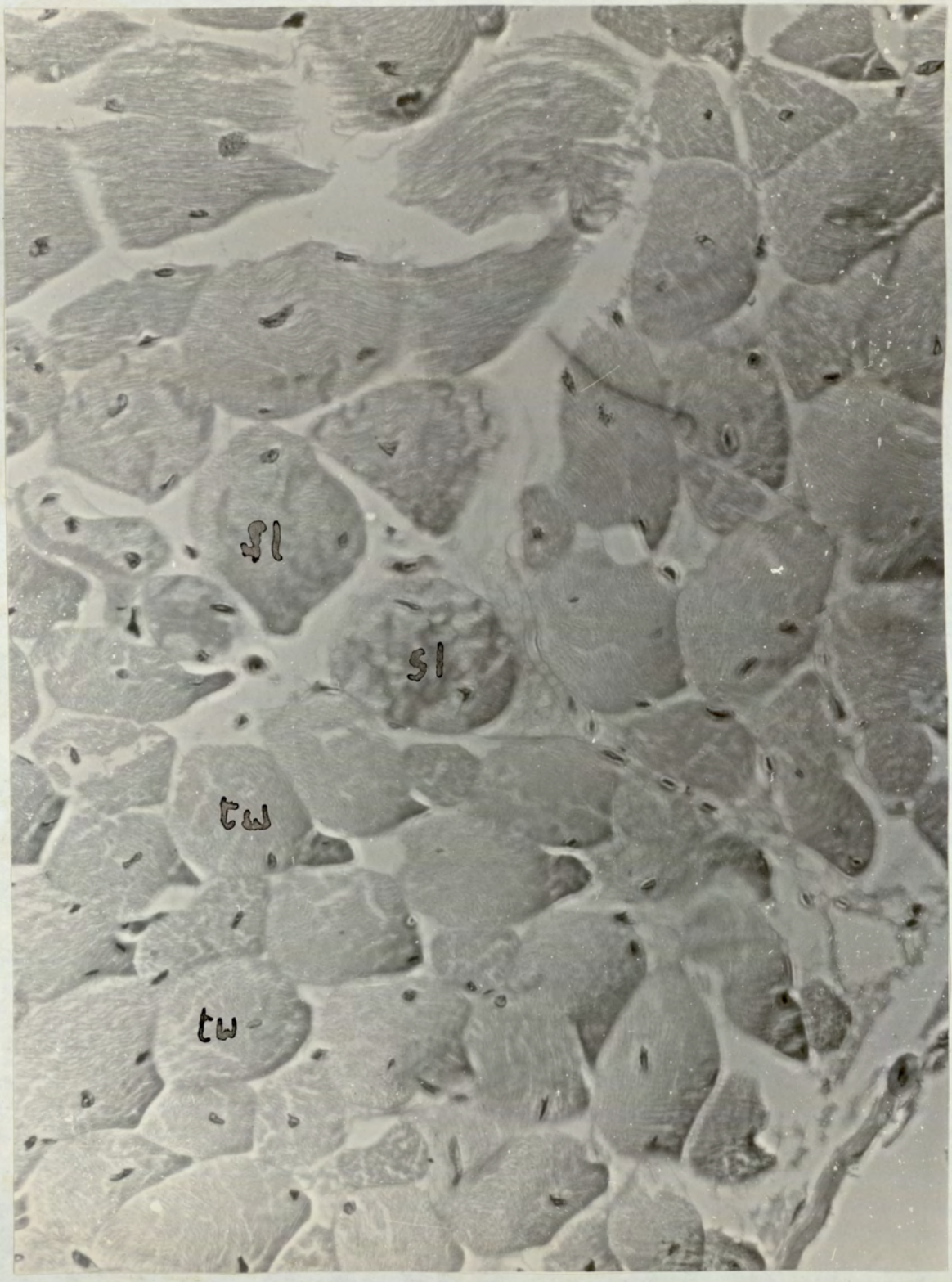


Plate 5: Tibialis anticus of frog (Rana temporaria). T.S. through middle portion, x 410. sl = slow fibre; tw = twitch fibre.

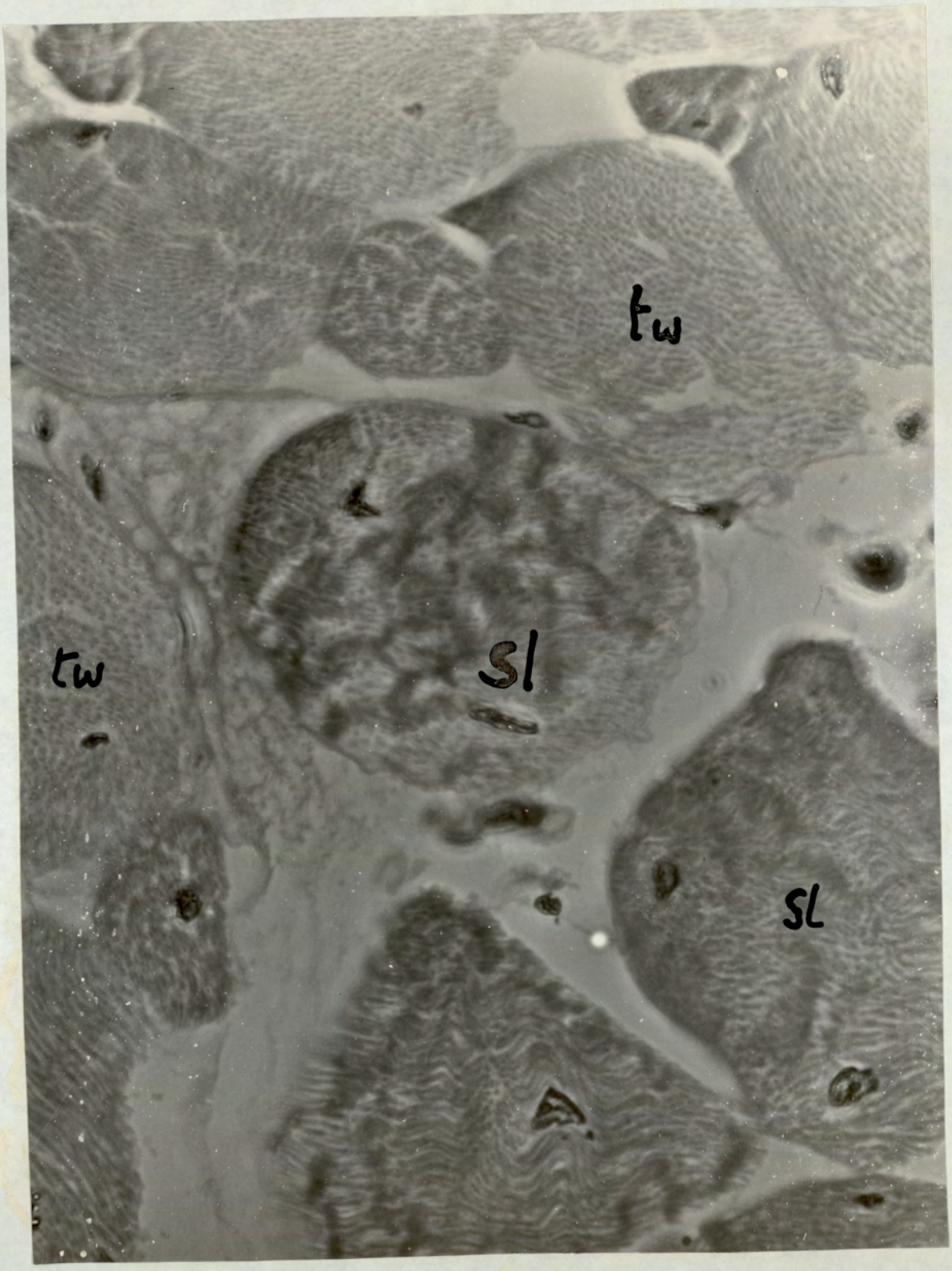


Plate 6: Tibialis anticus of frog (Rana temporaria). T.S. through middle portion, x 1040. sl = slow fibre; tw = twitch fibre.

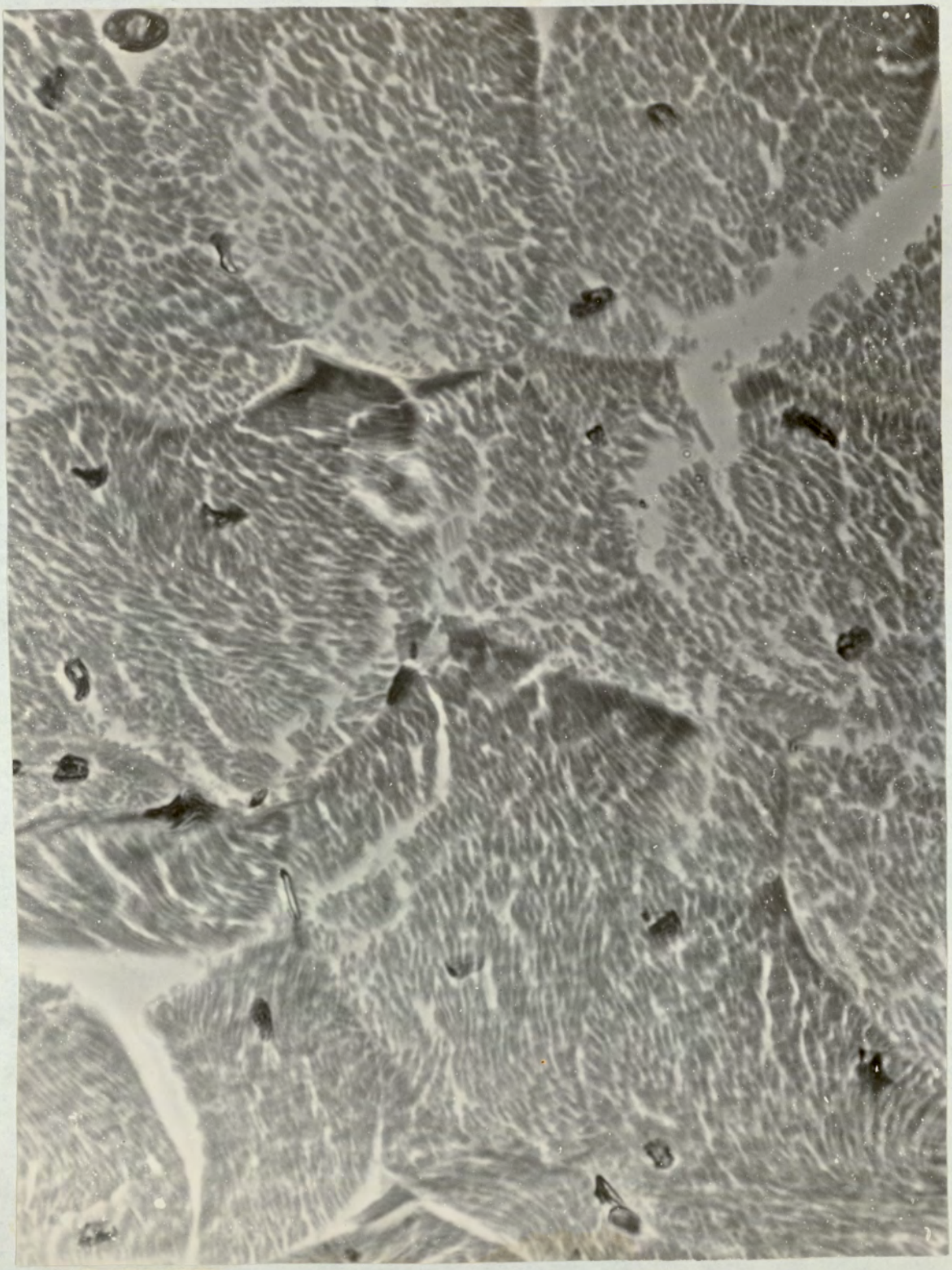


Plate 7: Peronaeus longus of frog (Rana temporaria). T.S. through middle portion, x 1,040. All fibres are of twitch type.

DISCUSSION: The distinction between fast and slow muscle fibres in the frog has been largely based on their physiological and pharmacological properties. However, Gray (1958) has illustrated and described the structure of sartorius, a purely tetanic muscle, and of extensor longus digitorum IV and adductor magnus, both of which contain both fibrillar- and areal-patterned fibres. Peachey and Huxley (1962) illustrated the structure of the tonus bundle of iliofibularis, containing both types of fibre.

The results described here demonstrate that peronaeus longus in the frog (Rana temporaria) consists exclusively of twitch fibres and belongs to Sommerkamp's (1928) group (a), and that tibialis anticus contains both fibrillar- and areal-patterned fibres and belongs to Sommerkamp's group (b). Probably it is because the muscles were allowed to shorten during fixation that the slow fibres show the appearance of containing ribbon-like associations of fibrils, well seen in Plate 6, similar to the structure described for muscle fibres of the sea horse (Hippocampus) by Rollett (1888) and Veratti (1902).

A point of interest is whether evidence can be adduced to support the proposition that the twitch fibres of the flexor, subserving a rapid withdrawal, might differ from the twitch fibres of the extensor, employed for thrust. On the basis of optical microscopy, it merely emerges that the twitch fibres of the flexor are of smaller diameter. A. V. Hill (1950) has pointed out that the muscles of small animals tend to contract more rapidly than those of larger animals, so that a small diameter does not preclude more rapid contraction.

In the mammal, fibres with areal structure occur in all the extraocular muscles, as demonstrated in the cat by Hess and Pilar (1963) but some other basis must be sought for the distinction between slow (red) and rapid (white) mammalian skeletal muscles.

B. ELECTRON MICROSCOPY

Peachey and Huxley (1962) have illustrated the structure of slow and twitch fibres in the semitendinosus of the frog; Adrian and Peachey (1965), in the tonus bundle of iliofibularis, and Page (1965), in the extensor longus digitorum IV. In each case, slow fibres have been compared with associated twitch fibres. Page and Huxley (1963) included frog sartorius, a purely tetanic muscle, together with semitendinosus and extensor longus digitorum IV (both of which contain both slow and twitch fibres), but a direct comparison between twitch fibres from a purely tetanic muscle with those interspersed with slow fibres has not been made. Since no account was available of the electron microscopic structure either of peronaeus longus or of tibialis anticus of the frog, longitudinal and transverse sections were prepared and examined.

METHOD: A frog (Rana temporaria) was killed. Peronaeus longus and tibialis anticus were removed, pinned, at their resting lengths, in troughs cut in wax, and slimmed down. The muscles were fixed in 25% glutaraldehyde (1 part) and 0.5M sodium cacodylate buffer (4 parts) for 3 hours; transferred to equal parts of 0.44M sucrose and 0.2M phosphate buffer, and stained with Caulfield (1957) osmium tetroxide. The muscle specimens were embedded in Araldite and sectioned on an LKB ultramicrotome.

RESULTS: The results are illustrated in Plates 8 - 13. Additional photographs were examined and used to obtain the data for Tables 1 and 2.

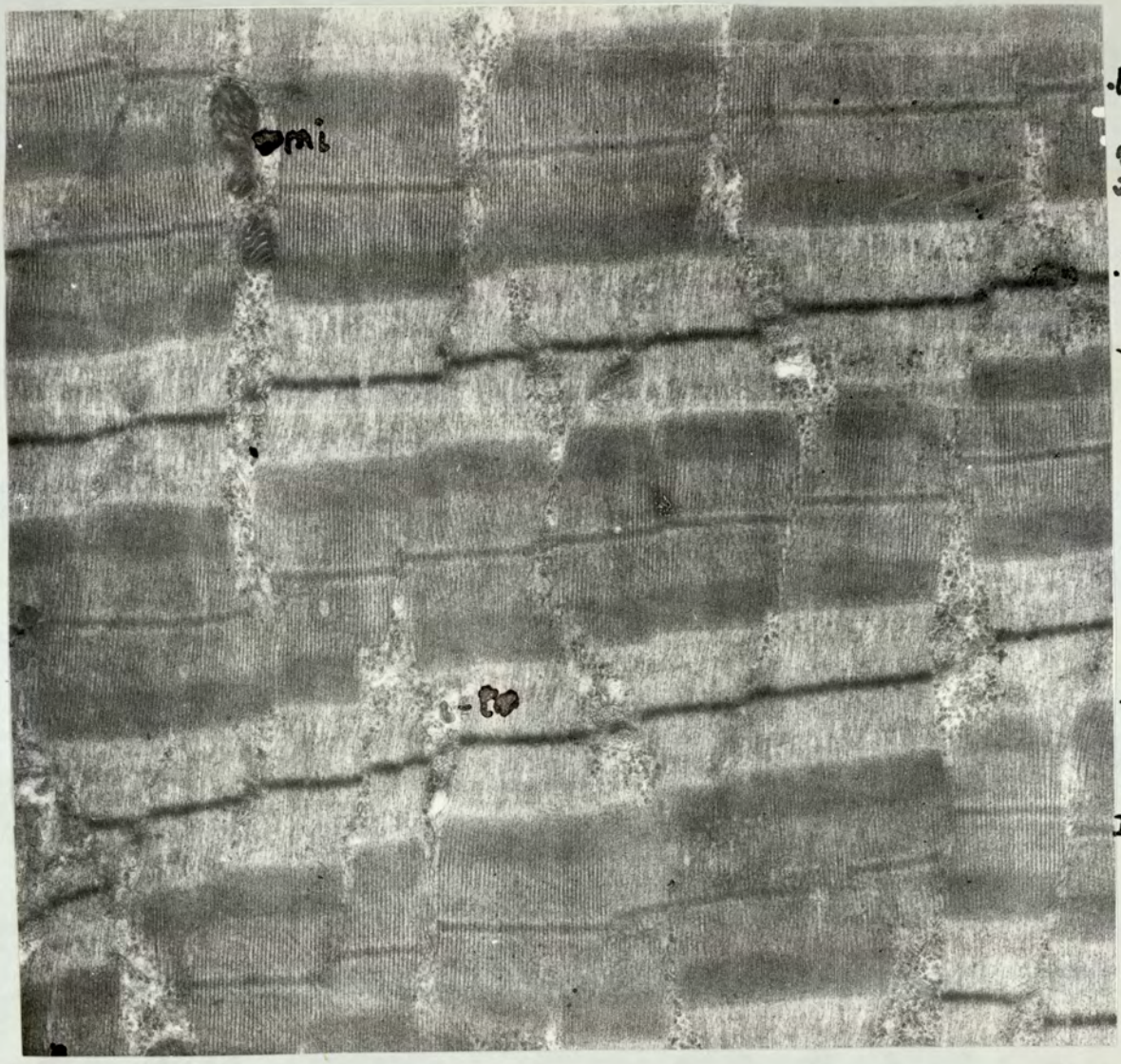


Plate 8: Electron micrograph of longitudinal section of a twitch fibre from peronaeus longus of frog (Rana temporaria) x 16,000.

tr = triad; mi = mitochondrion; M = M line; Z = Z line; A = anisotropic zone; I = isotropic zone; H = central part of A zone, into which actin filaments do not extend; L = L zone, where myosin filaments are bare; overlap is of actin and myosin filaments.

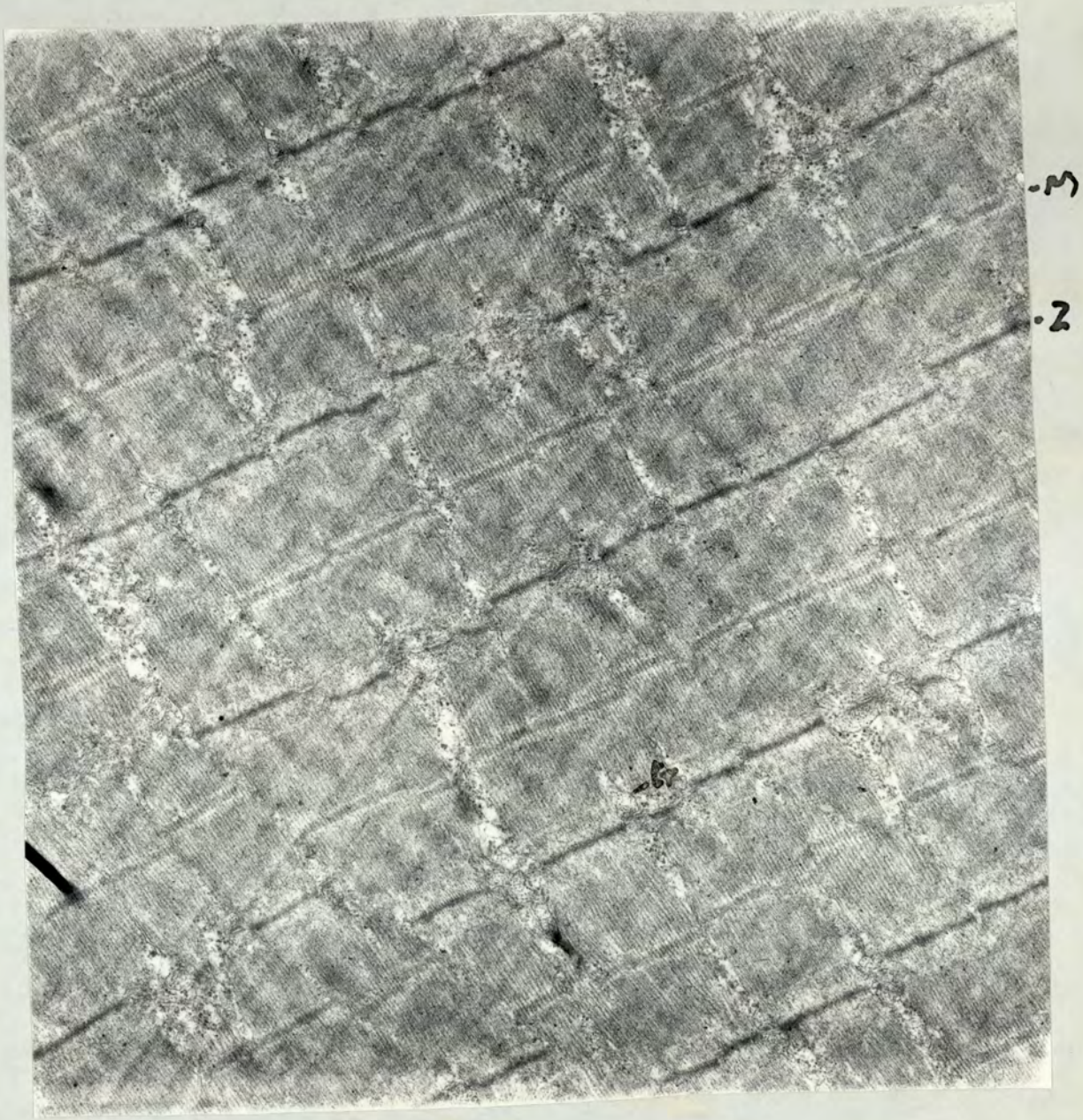


Plate 9: Electron micrograph of longitudinal section of a twitch fibre from tibialis anticus of frog (Rana temporaria), x 16,000. tr = triad; M = M line; Z = Z line.

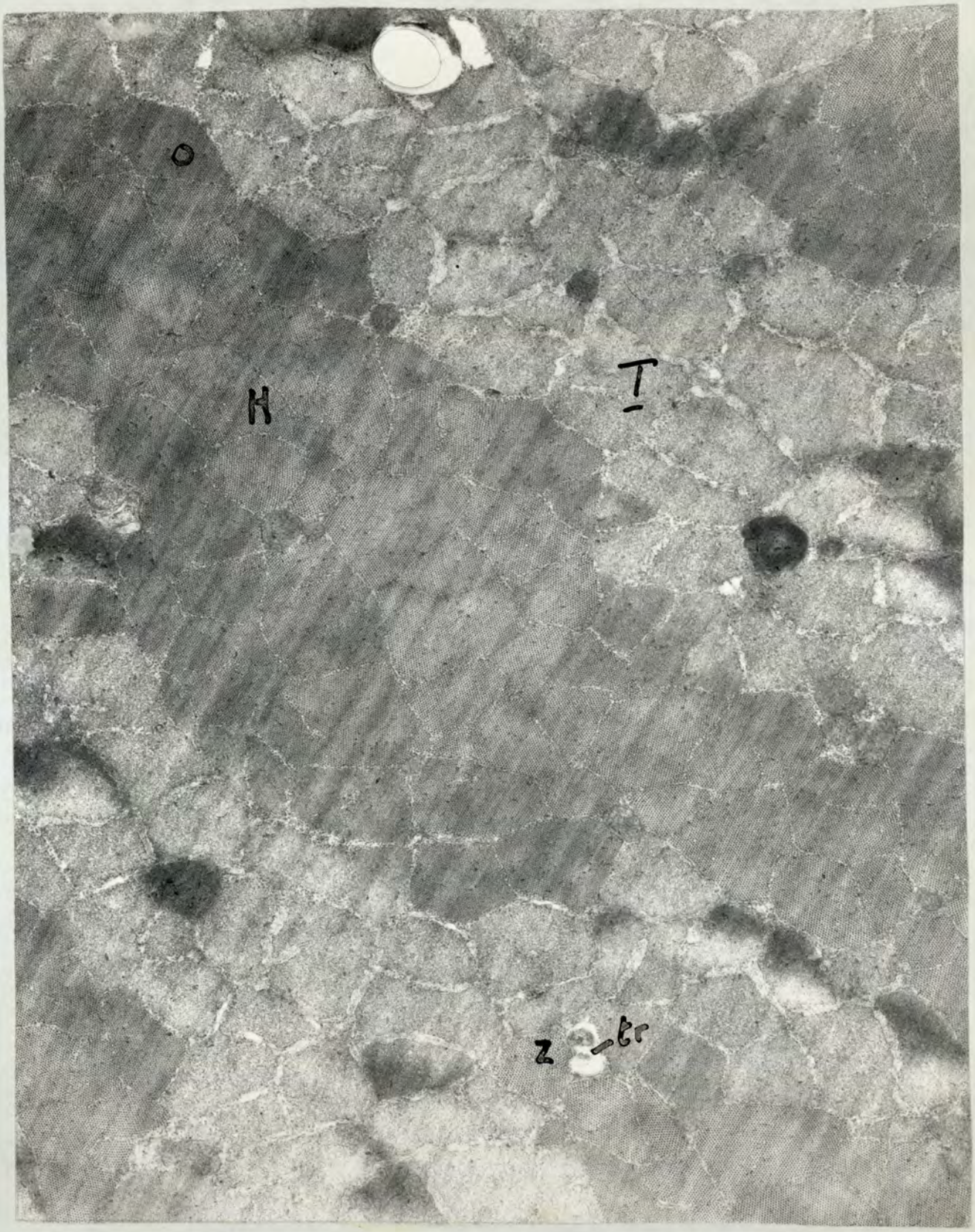


Plate 10: Electron micrograph of transverse section of a twitch fibre from peronaeus longus of frog (Rana temporaria), x 16,000. H = H zone; I = I zone; O = overlap region of A zone. tr = triad, Z = Z line.

Plate 11: Electron micrograph of transverse section of a twitch fibre from tibialis anticus of frog (Rana temporaria), x 16,000.

H = H zone; I = I zone; tr. = triad; t = transverse tubule; mi = mitochondrion.

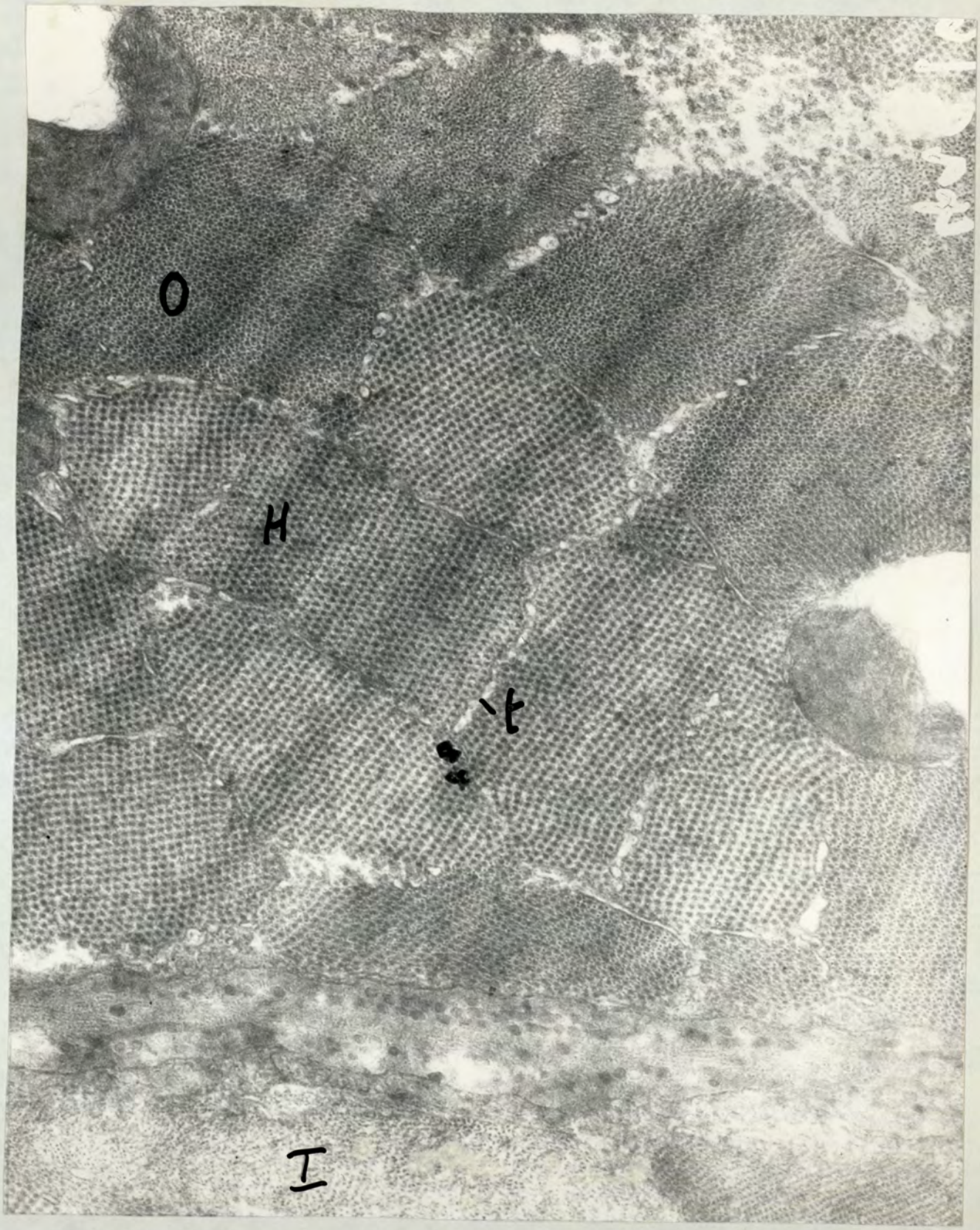


Plate 12: Electron micrograph of transverse section of a fibre from peronaeus longus of frog (Rana temporaria), x 64,000.

H = H zone; I = I zone; t = transverse tubule; O = overlap of actin and myosin filaments.

Plate 13: Electron micrograph of transverse section of a twitch fibre from tibialis anticus of frog (Rana temporaria), x 64,000. H= H zone; I = I zone; O = overlap of actin and myosin; Z = Z line; tr = triad; mi = mitochondrion.

The dimensions of the constituent parts of the myofibrils of fibres from peronaeus longus and twitch fibres of tibialis anticus, obtained from photographs of longitudinal sections, are set out in Table 1.

	Peronaeus longus	Tibialis anticus
	µm	µm
Sarcomere length	3.3 ± 0.06 (22)	2.4 ± 0.06 (40)
Length of A filament	1.9 ± 0.01 (45)	1.6 ± 0.06 (45)
Length of I filament	2.2 ± 0.01 (50)	1.2 ± 0.06 (22)
Length of H zone	1.0 ± 0.06 (51)	0.9 ± 0.06 (22)
Length of I zone	1.4 ± 0.06 (51)	0.6 ± 0.06 (40)
Length of overlap (A - H)	0.9 (means)	0.7 (means)
Length of L zone	0.19 (approx.)	0.21 (approx.)
Thickness of Z line	0.09 (approx.)	0.06 (approx.)
Thickness of M line	0.06 (approx.)	o.06 (approx.)

TABLE 1: Dimensions of constituent parts of myofibrils of twitch fibres from peronaeus longus and tibialis anticus of the frog (Rana temporaria). S.E. of the Mean, with number of measurements in brackets.

Triads are seen at the level of the Z line in both peronaeus longus (Plate 8) and tibialis anticus (Plate 9).

The mean diameter of the myofibrils of the fibres of peronaeus longus is 0.7 ± 0.1µm (Plate 10), and of twitch fibres in tibialis anticus is 0.4 ± 0.1µm (Plate 11).

From high magnifications (x 64,000) of the region of overlap in the A band, it can be seen that, in twitch fibres of both peronaeus

longus (Plate 12) and tibialis anticus (Plate 13), each myosin filament is surrounded by six actin filaments, and each actin by three myosin filaments; thus, there are twice as many actin filaments as there are myosin filaments, and the muscles do not differ in this respect. In transverse sections, as in longitudinal, the region of overlap is indicated as being shorter in tibialis anticus than in peronaeus longus, since few such areas are to be found on the photographs of the former prepared here.

DISCUSSION: The striation pattern of skeletal muscle is due to differing degrees of birefringence occassioned by the arrangement of two interdigitating sets of filaments, thick and thin respectively, within the myofibrils. Typically, the thick filaments are about 110 Å in diameter and 1.6 µm in length (Page, 1964), are slightly thickened at the centre to form the M line, and run the length of the anisotropic A band. The thin filaments arise from the Z lines, where each I filament faces the space between four I filaments on the opposite side of the Z line, and these are interconnected by four Z filaments (Knappeis and Carlsen, 1962). The thin filaments are about 50 Å in diameter and their overall length, including the Z line, is about 2.05µm. in the frog (Page, 1964) and 2.35µm. in the mammal (Elliott, Lowy and Worthington, 1963; Page and Huxley, 1963); they extend through, and beyond, the isotropic I band, to overlap the thick filaments in the A band. The H zone is that part of the A band into which the I filaments fail to penetrate (H. E. Huxley and Hanson, 1954; Hanson and Huxley, 1955). The thick filaments are covered by projections, which in the overlap region may attach to receptive points on the thin filaments. There are no projections in the middle of the thick filaments, hence, a light

region, bisected by the M line, is seen and is known as the L zone; it is 0.15 to 0.20μm. long (H. E. Huxley, 1957).

	Purely tetanic muscle			Twitch fibres associated with slow								Slow fibres		
	Peronaeus longus Table I	Sartorius Peachey (1965)	Sartorius Page and Huxley (1963)	M E A N	Tibialis anticus Table I	Semitendinosus Muscatello, et al (1961)	Semitendinosus Peachey and Huxley (1962)	Semitendinosus Muscatello, et al (1965)	Extensor longus digitorum IV Page (1965)	Ilio fibularis (tonus bundle) Adrian and Peachey (1965)	M E A N	Iliofibularis (tonus bundle) Adrian and Peachey (1965)	Iliofibularis (tonus bundle) Peachey and Huxley (1962)	Extensor longus digitorum IV Page (1965)	M E A N
Sarcomere length	3.3	2.9	3.2	3.1	2.2	3.3	2.2	2.0	2.5	2.4	2.4	3.5	1.9	2.5	2.6
Length of A filament	2.0	1.6	1.5	1.7	1.7	1.4	1.2	1.1	1.2	1.3	1.3	1.0	1.2	1.2	1.1
Length of I filament	2.1	1.9	1.9	2.0	1.3	2.3	1.3	1.3	1.7	1.4	1.6	3.1	1.2	1.7	2.0
Length of H zone	1.1	1.0	1.1	1.1	0.9	0.8	0.7	0.7	0.8	1.0	0.8	0.3	0.3	0.8	0.5
Length of I zone	1.4	1.3	1.5	1.4	0.7	1.9	0.8	0.9	1.2	1.1	1.1	2.2	0.7	1.2	1.4
Length of overlap (A-H)	0.4	0.3	0.2	0.3	0.1	0.3	0.2	0.4	0.4	0.3	0.3	0.7	0.5	0.4	0.5
Length of L zone	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	-	0.1	0.1	-	-	-	-
Thickness of Z line	0.06	0.03	0.06	0.05	0.03	0.05	0.04	0.04	0.05	0.03	0.04	0.09	0.08	0.05	0.07
Thickness of M line	0.03	0.06	0.06	0.05	0.03	0.05	0.04	0.04	-	0.03	0.04	-	-	-	-

TABLE 2: A comparison of the dimensions of the myofibrillar components in muscles of the frog. The length of the I filaments tabulated is the overall length, including the Z line. All measurements are in um.

In Table 2, the mean dimensions obtained for peronaeus longus and tibialis anticus (Table 1) are incorporated with measurements taken from photographs in the cited literature of a variety of muscle fibres. It should be noted that, during fixation with 1% osmium tetroxide, the I filaments may shorten by 10%, and the A filaments by 6 to 7%. Glutaraldehyde may cause a 2 to 3% shortening of the I filaments, but little of the A (Page and Huxley, 1963). No great reliance can be placed on the data, in view of the small number of examples and variations in the fixation procedure, but they suggest that the sarcomere, thick filaments, and thin filaments are longer in the fibres of a purely tetanic muscle than in twitch fibres associated with slow fibres

From the generally accepted sliding filament theory (H. E. Huxley and Hanson, 1954; A. F. Huxley and Niedergerke, 1954) it would be expected that, the greater the number of cross-bridges, the greater would be the tension developed by the muscle; and, the fewer the cross-bridges, the faster the contraction of the muscle. Were this the only factor, one might predict that a fibre in a purely tetanic muscle would have a greater overlap in the A zone than a twitch fibre associated with slow fibres, as the former produces a powerful thrust, and the latter a rapid withdrawal. While there is evidence for this to be seen in Table 1, it is not confirmed in Table 2.

The thick filaments consist of myosin, the thin filaments are of actin, and some other material forms the Z filaments (Hanson and Huxley, 1953, 1955). The projections on the thick filaments are thought to arise in pairs from opposite sides of the myosin filaments every 143 $\mathring{A}$, with a rotation of 120° between each successive pair, so that projections orientated in the same direction occur every 429$\mathring{A}$ (H. E. Huxley and Brown, 1967). This is similar to the 435$\mathring{A}$ repeat distance estimated by Worthington (1959) on the basis of the X-ray diffraction pattern of

living muscle. The actin filament consists of two helices, with cross-over points spread at intervals of 349Å (Hanson and Lowy, 1963), which does not accord with the 406Å periodicity observed in I fibrils in the electron microscope defined by Page and Huxley (1963). The lengths of the A and I filaments remain constant, being the same in both resting and active muscles at all sarcomere lengths above 2.1μm., below which, the I filaments may be compressed (Page, 1964; H. E. Huxley, Brown and Holmes, 1965; Elliott, Lowy, and Millman, 1965). Gordon, Huxley, and Julian (1966) showed the relation between sarcomere length and isometric tension developed in isolated twitch fibres from semitendinosus of the frog. Tension fell to zero at sarcomere lengths close to 3.65μm., where overlap between thick and thin filaments is expected to cease; there was a plateau of tension from 2.2 to 2.0μm., at which length each thin filament just overlaps all the bridges at the far end of the thick filaments; below the plateau, tension fell gradually with decreasing sarcomere length to about 1.65μm., at which point the ends of the thick filaments come into contact with the Z line; finally, there was a steep decline in tension, to reach zero at about 1.3μm.

Because of the interdigitation of the filaments, almost the whole of the change in length occurs within the I bands. A. F. Huxley and Niedergerke (1954), using isolated fibres of frog muscle, observed the change in length of the I band during release, passive stretch, and during an isotonic twitch. There was no change in the length of the bands during an isometric twitch, unless the fibre in fact shortened. H. E. Huxley and Hanson (1954) showed that, in separated myofibrils of frog muscles, likewise, it was only the I bands which changed in length when released, stretched, or caused to contract by adenosinetriphosphate (ATP).

The myofibrils are surrounded by a complex of tubules, enlarged in places to form cisternae, the sarcoplasmic reticulum. Its form and distribution in the sartorius and cardiac muscle of the rat were described by Porter and Palade (1957); in the carpopedal extensor of the crab by Peachey and Huxley (1964), and in the sartorius of the frog by Peachey (1965). In the frog, Peachey (1965) described a system of longitudinal tubules surrounding the A band, with a fenestrated collar in the region of the M line. It seems likely that the longitudinal tubules are interconnected to form a network, and that the fenestrated collar represents sections through the connecting tubules. The longitudinal tubules arise from cisternae, the terminal cisternae, in the vicinity of the Z line. Other dilatations of the longitudinal plexus form intermediate cisternae at other levels. Between, and in close contact with, two terminal cisternae is a transvers tubular system, or T system. The longitudinal tubules are thin-walled, the transverse tubules are thick-walled (Nelson and Benson, 1963); Franzini-Armstrong and Porter, 1964). Franzini-Armstrong and Porter (1964) estimated that there were 20 to 30 openings at each Z line level around the circumference of a 12μm.-diameter fish muscle fibre. Adrian, Costantin and Peachey, (1968), estimated the length constant of the transverse tubules, for fibres with diameters of 47 to 145μm., in frog semitendinosus, as being between 50 and 90μm., each tubule thus being much longer than the diameter of a single myofibril (c.f. Plate 11). In the crab, (A. F. Huxley and Taylor, 1958; Peachey and Huxley, 1964); lizard (A. F. Huxley and Straub, 1958); mouse (Andersson-Cedergren, 1959), and rat (Walker and Schrodt, 1965) the triads, i.e. groups of terminal cisternae and transverse tubules, are in the region of the A-I junction, and not at the more usual Z line site.

Veratti (1902) originally described a transverse reticulum coincident with the Z line, joined to that of the adjacent sarcomere by sparse long filaments. Porter and Palade (1957) similarly described the T system as a series of vesicles. That the transverse tubules are continuous, has been established in the muscles of the fish (Fawcett and Revel, 1961); bat (Revel, 1962), and mouse (Andersson-Cedergren, 1959). A. F. Huxley (1964) immersed frog sartorius muscles in ferritin (M.Wt., 460,000) and demonstrated granules in the transverse tubules only. This indicated that the T system is in open communication with the extracellular space. Karnowsky (1965) used horseradish peroxidase as an extracellular marker to show that this passed from cardiac capillaries into the transverse tubules. Franzini-Armstrong and Porter (1964), using fish muscle, stressed that there was no direct connection between the T system and the other components of the sarcoplasmic reticulum. Endo (1966) showed that a fluorescent dye entered the T system of single fibres of the frog semitendinosus muscle. D. K. Hill (1964) found, that when sartorius muscles of the toad were soaked in a solution containing tritium-labelled albumin and fixed in osmium tetroxide or freeze-dried, about half the albumin space is at the level of the Z line, the rest in a pair of spaces near the A-I boundary. It is possible that the results of Endo (1966) and D. K. Hill (1964a) reflect a different arrangement of the sarcoplasmic reticulum as between a muscle containing slow fibres and a purely tetanic muscle. This hypothesis need not be at variance with the finding of A. F. Huxley (1964), since the unidentified structure of D. K. Hill (1964a) near the A-I junction could be permeable to albumin and not to ferritin.

By observing single fibres of frog semitendinosus muscles by polarized light and placing a capillary electrode at various points on the surface, A. F. Huxley and Taylor (1955) showed that it was only when the neighbourhood of the Z line was stimulated that the I band shortened. Repetition of this experiment with the muscles of the green lizard (A. F. Huxley and Straub, 1958) and crab (A. F. Huxley and Taylor, 1958), proved that half an I band could be made to contract, pulling the Z line towards it, when a stimulus was applied near the A - I boundary. These experiments led to the view that there is an inward spread of conduction in muscle transmitted through the T system, since this system is located at the Z line in the frog, and at the A - I boundary in the lizard and crab. Gonzales-Serratos (1966) estimated the rate of inward conduction in single fibres from semitendinosus of the frog as 3cm./sec. at 5°C.; 4cm./sec. at 10°C., and 8cm./sec. at 20°C. Peachey (1965) suggested that the transverse tubules were electrically coupled to the terminal cisternae, so that depolarization of the fibre surface spreads inwards along the T system to the terminal cisternae, initiating the release of a contraction-activating substance. D. K. Hill (1964b), using toad sartorius, showed that a large proportion of the adenosine nucleotide, which is concentrated in the I band, lies in the interfibrillar spaces, rather than in the fibrils themselves. Being shown as lying in rows of vesicles lined up transversely in the I band, the adenosine nucleotide is clearly associated with the T system and could represent the contraction-activating substance, a precursor, or co-agent.

The electron microscopic study of the muscles under review, combined with a re-examination of available published material,

does not permit a firm conclusion to be drawn. Further studies, giving particular attention to the constituents of the sarcomere and to the arrangement of the sarcoplasmic reticulum, are warranted. Hess (1970) maintains that there is only one type of twitch fibre, yet insists that the nerve-endings in purely tetanic muscle fibres are of the typical end-buschel form, whereas twitch fibres associated with slow fibres had nerve-endings which were shorter, more branched, and more variable in appearance than the Endbuschel.

CONCENTRATION RELATIONS OF POTASSIUM AND SODIUM

Hodgkin and Horowicz (1960) suggested that the potassium conductance might be located in the tubular membrane of the T system. Freygang, Golstein and Hellam (1964) carried out intracellular recordings of the negative after-potential that follows a train of impulses in frog sartorius muscles and postulated that an accumulation of potassium during the train, in a space located between the major portion of the sarcoplasm and the external fluid, caused this after-potential. The decline of after-potential would, in that case, be produced by the exchange of potassium from the intermediate space with sodium from the external fluid. Freygand, et al. (1964) found that dilatation of the T system occurred both in hypertonic and in low chloride Ringer, whereas in potassium-free Ringer there was a small change in the opposite direction. The late after-potential was prolonged both in hypertonic and low chloride Ringer, and shortened in a potassium-free Ringer.

Fatt (1964) analysed the transverse electrical impedence of frog sartorius. Falk and Fatt (1964) suggested that the membrane of the transverse tubules increased the capacitance of muscle fibres. Adrian and Peachey (1965), using the tonus bundle of frog iliofibularis, found that the membrane capacity per unit surface area of the twitch fibres was about three times as great as that of slow fibres. The membrane resistance of slow fibres appeared to be about ten times as great as that of fast fibres. Winegard (1965)autoradiographing frog toe muscles soaked in Ca^{45} and fixed with an osmium oxalate solution, found a majority of grains over the A band coincided with the fibrils, The grain density over the I bands was greatest over the space between the myofibrils.

He deduced, therefore, that all, or most of, the calcium in the A band is within the fibrils; most of the I band calcium between the fibrils. He suggested that, in the relaxed state, exchangeable calcium was located primarily in the lateral cisternae of the sarcoplasmic reticulum, and, in the contracted state, it was in the myosin filaments.

Eisenberg and Gage (1967) found that, in the frog sartorius, destruction of the T system, by immersion in glycerol Ringer and transfer to normal Ringer, markedly reduced membrane capacitance and prevented the slow, progressive increase in potential, known as 'creep', which normally follows the application of hyperpolarizing current pulses. Gage and Eisenberg (1967) likewise found that the after-depolarization following a single action potential, and the slower late after-potential following a train of action-potentials were absent after disruption of the T system. Using the peroxidase method, it has been estimated that only 2% of the transverse tubular system persists after soaking frog sartorius muscles in glycerol Ringer for one hour and returning to normal Ringer (Eisenberg and Eisenberg, 1968). Eisenberg and Gage (1969) have deduced that chloride conductance is located only on the surface membrane, whereas potassium conductance is distributed between the tubules and the surface membrane. Stefani and Steinbach (1968), using the frog iliofibularis, found that, in whole muscles, a change from glycerol Ringer to normal Ringer abolished the phasic contraction produced by a high concentration of potassium ions, due to the twitch fibres, but that the maintained contracture, due to the slow fibres, persisted.

Differences in the functioning of muscles might be linked with characteristic ionic distribution so the ratios of potassium-to-sodium in the fibre water of the muscles under review were studied. Adrian (1960)

drew attention to the fact that the largest uncertainty in any estimate of the intracellular concentration of an ion in muscle is introduced by the intercellular space and, as a guide to this determined the dry-to-wet- weight ratio of the frog sartorius. Similar determinations have, therefore been made here.

METHOD: The method described by Adrian (1956) was used with minor variations: The muscles were carefully removed and, after blotting with ashless filter paper (Whatman No. 542) were suspended from the arm of a 100mg. torsion balance by about 1cm. of fine cotton thread tied to the tendon. After weighing, each muscle was hung from a pin in the cork of a specimen tube and dried overnight in an oven at 80°C. The dry muscle, with its thread, was weighed and, after cutting the muscle from its thread, the dry and wet weights of the thread were found. The dry ~~and~~ to wet weights ratio of the muscle alone ~~were~~ was expressed as a percentage. The dry muscles, in platinum crucibles, were put into a furnace overnight at 520 - 580°C. The ash was washed into Pyrex volumetric flasks, and the resulting solution was compared in an EEL flame photometer with a standard containing 100µmole/l. KCl and 50µmole/l. NaCl. Volumetric flasks of 50 or 100ml. capacity were used to bring the final potassium concentration reasonably close to that of the standard. The method of calculation is given with the results.

RESULTS: The ratio of dry-weight-to-wet-weight in tibialis anticus and in peronaeus longus was determined for both limbs of eight frogs. The results are given in Table 3:

Tibialis anticus		Peronaeus longus	
%	%	%	%
18.5	15.2	16.2	16.7
18.5	18.5	18.2	16.7
14.9	16.1	16.7	17.6
18.1	20.3	17.8	17.3
17.6	19.4	20.2	20.9
18.8	20.5	19.0	19.5
18.6	19.3	17.6	17.1
18.9	18.0	16.5	18.2
Mean = 18.2 ± 0.4		Mean = 17.9 ± 0.4	

TABLE 3: Ratio of dry-weight-to-wet-weight in tibialis anticus and peronaeus longus of the frog (Rana temporaria). Standard errors of the Mean are given. Both legs from eight frogs were used

In calculating the concentrations of potassium and sodium in the fibre-water (C_{fw}, in m.mole/kg. fibre water), the formula used was that given by Adrian (1956):

$$C_{fw} = \frac{C_m - 0.125.C_o}{1 - (\text{Dry wt.} + \text{Wet wt.}) + 0.125}$$

where C_m is the concentration of metal in the muscle, and C_o that in the surrounding fluid, both expressed in m.mole/kg. These results are given in Table 4:

	Tibialis anticus			Peronaeus longus		
	$C_{fw}K$	$C_{fw}Na$	K/Na	$C_{fw}K$	$C_{fw}Na$	K/Na
(a) Muscles taken directly from animals:						
	110.2	32.8	3.36	90.3	13.9	6.50
	118.5	25.9	4.58	110.2	14.8	7.45
	127.3	42.7	2.98	95.8	21.5	4.46
	136.5	38.3	3.56	135.6	38.7	3.50
	123.7	35.6	3.47	118.6	26.0	4.56
	131.2	30.0	4.37	133.4	29.6	4.51
Mean	124.6 ± 1.6	34.2 ± 1.0	3.72 ± 0.1	114.0 ± 3.1	24.1 ± 1.6	5.16 ± 0.25
(b) After 1 hour in frog Ringer:						
	108.4	109.1	0.99	116.7	84.4	1.38
	116.3	76.2	1.53	127.4	69.9	1.82
	94.8	65.5	1.45	101.1	45.5	2.22
	99.2	58.7	1.69	95.5	45.2	2.11
	102.8	49.6	2.07	105.6	47.8	2.21
	98.3	49.0	2.01	114.9	46.4	2.48
Mean	103.3 ± 1.3	68.0 ± 9.1	1.62 ± 0.07	110.2 ± 1.9	56.5 ± 2.8	2.04 ± 0.06
(c) After 4 hours in frog Ringer:						
	94.2	49.3	1.91	73.5	40.9	1.80
	98.8	49.2	2.01	74.1	24.3	3.05
Mean	96.5 ± 1.6	49.3 ± 0.05	1.96 ± 0.04	73.8 ± 0.21	32.6 ± 5.8	2.42 ± 0.44

Table 4: Ratios of potassium to sodium in frog muscles (Rana temporaria). Ionic concentrations in m.mole/kg. Standard errors of the mean.

In experiments where the muscles were taken directly from the animal for analysis, the value of C_o was based on frog plasma (Conway, 1957), viz.: for potassium, 2.15mmole/kg., and for sodium, 103.8mmole/kg.

DISCUSSION: Using 18 frog sartorius muscles, Adrian (1960) obtained a dry-to-wet-weight ratio of 16.6 ± 0.25%. The values obtained here for tibialis anticus and peronaeus longus, using 16 muscles in each case (Table 3), are slightly higher. They cannot be regarded as differing significantly from each other.

Estimates of intracellular concentrations of potassium and sodium in frog sartorius have been made by previous workers. Conway (1957) gives the mean value from eleven authors (loc. cit.) as 83.8mmole/kg. potassium and 23.9mmole/kg. sodium. Desmedt (1953) found the mean concentration for nine short-soaked muscles to be 97 ± 2mmole/kg. potassium and 23.9 ± 1.4mmole/kg. sodium; for four muscles soaked for four hours in phosphate Ringer, the same author found the means to be 150mmole/kg. potassium and 20mmole/kg. sodium. Adrian (1960) estimated the internal potassium concentration of 14 muscles which had been soaked in normal frog Ringer, and obtained a mean value of 139 ± 2mmole/kg. The differences between the values arrived at are in part due to the use of Ringer solutions of different compositions, to soaking for varying periods, and to employing differing divisors in the equation on which the calculations are based. The last factor, presumably, arose from different degrees of drying, and an allowance by Desmedt (1953) for a change in the proportion of intracellular space after prolonged immersion.

The results obtained for the muscles under review (Table 4) are of the same order as for sartorius. Peronaeus longus has a higher potassium-to-sodium ratio than tibialis anticus, and this obtains whether the muscle is taken directly from the animal, or after immersion in Ringer for a shorter or longer period. After prolonged immersion, fluid drips from the muscles, probably from the connective tissue, and

the ratios approach the original values, as might be predicted. The deposit in the specimen tubes in which peronaeus longus had been dried after prolonged immersion was visibly greater than in the case of tibialis anticus.

During immersion, the concentration of potassium falls progressively, whereas the concentration of sodium approximately doubled in one hour and declined thereafter. Fenn (1936), reviewing electrolytes in muscle, refers to a loss of potassium and a gain in sodium by frog sartorius in Ringer.

In view of the fact that the proportion of slow fibres in tibialis anticus is small, differences in ionic concentrations between this and a purely tetanic muscle are likely to reflect differences between the twitch fibres in the two types of muscle. On the simple potassium electrode hypothesis, the resting potentials of the twitch fibres in the two muscles at 18°C., using the formula:

$$E = 58 \log_{10} \frac{[K]_o}{[K]_i}$$

would be: Peronaeus longus, 100.0mV; tibialis antichus, 102.3mV.

THE DEVELOPMENT OF EXPERIMENTAL TECHNIQUES

The primary inducement to embark on these investigations was a desire to increase the range and usefulness of student exercises in pharmacology. In particular, the practice of carrying out physiological experiments on the frog sciatic-gastrocnemius, and pharmacological exercises on the frog rectus abdominis preparation led to some confusion. The rectus abdominis is less obviously a voluntary and skeletal muscle than the gastrocnemius, and it is important to reinforce the concept of pharmacology as applied physiology. Accordingly, the earliest experiments were performed on the frog sciatic-gastrocnemius preparation in a Keith Lucas muscle bath, wherein drugs can be applied in known concentration, stimulating the nerve by means of a du Bois Reymond inductorium, and recording on a smoked drum in the traditional manner. Within a month of the first experiment, an interest in the possibility of antagonistic muscles responding differently led to the use of a sciatic-tibialis anticus preparation, and finally to a double nerve-muscle preparation, consisting of the sciatic nerve together with tibialis anticus and peronaeus longus. Discussing the results of the first year's work, Dr. I. C. Whitfield suggested that a neon tube would be preferable to an inductorium as a source of stimulation. A simple neon tube stimulator was made to his specification, later a Palmer electronic square-wave stimulator was obtained and, ultimately, to preclude objections to electrical means of stimulation, subcutaneous injections of convulsants were used. For recording the responses, a direct comparison of flexor and extensor was desirable, so a double myograph was designed for use with the Keith Lucas bath. The original model was made to specification by Palmers towards the end of 1954 and

carried tangential writing points. This was later modified to carry frontal writing levers, and eventually replaced by a pair of transducers.

METHODS:

I. PREPARATIONS

(a) The sciatic-gastrocnemius preparation: The traditional preparation, as described, for example, by Bell (1952) was used: A frog was pithed and a cut in the skin was made to encircle the body below the armpits. The skin was removed, in one piece, from the hinder part of the body. The frog was placed on a dissecting board on its belly, the hips flexed, and the urostyle cut away. The head of the frog was raised, a scalpel placed on the pubic symphysis and pressed down to divide the body between the two sciatic nerves. The vertebral column was transected, just in front of the origin of the sciatic nerves, its posterior end raised to enable the viscera and body wall to be cut away, without damage to the nerves. The two legs and connecting piece of vertebral column are turned to lie on the dissecting board with the ventral side uppermost, a scalpel placed along the midline, and struck sharply to bisect the vertebral column and stump of the urostyle. Each leg, in turn, is placed with the dorsal side uppermost and a 1½-inch pin pushed through the lower end of the femur, close to the knee. The sciatic nerve was exposed in the thigh, the sacroiliac joint divided and the sciatic nerve freed. A thread was tied round the Achilles tendon, above the sesamoid bone, and the tendon cut below that bone. The femur and tibiofibula were transected. The sciatic-gastrocnemius preparation was transferred to the Keith Lucas muscle bath.

(b) The sciatic-tibialis anticus preparation: On exactly similar lines, except that the tibialis anticus muscle was used.

(c) Isolated double nerve-muscle preparations: To record the contractions of tibialis anticus and peronaeus longus simultaneously, a frog is pithed and the skin removed from the posterior half of the body. The urostyle is removed, the pubic symphysis divided, and the lower vertebral column divided and bisected. A 1½-inch pin is driven through the lower end of the femur, and the gastrocnemius muscle reflected and cut away, care being taken not to damage the branches of the sciatic nerve to the remaining muscles of the leg. The sciatic nerve is dissected out as in (a) above. Peronaeus longus is separated from tibialis anticus, by dividing the connective tissue, and similarly freed from the tibiofibula. Tibialis anticus is separated from the bone as far as possible without damage to its nerve. A thread is tied round the tendon of insertion of each muscle. The free end of one thread may be looped to avoid confusion of the two muscles at a later stage. The lower end of the tibiofibula is separated from the tarsal bones, and its distal two-thirds cut away and discarded. Most of the foot is cut off. The tarsus is divided between the threads, so that a small portion of bone secures a thread to each muscle. The sciatic nerve is folded back, and the femur and thigh muscles divided close to the knee. The preparation is transferred to the muscle bath and attached to the myograph or transducers. Thin string is used to secure the pin in its platform and a short piece of string, in the form of a clove hitch, to press the knee against the platform. The Keith Lucas bath contains 150ml. frog Ringer which is aerated by an aquarium pump. Drops of liquid paraffin are run over exposed lengths of the sciatic nerve when this is placed over the electrodes.

The dissection and procedure where gastrocnemius is used with one of the other two muscles is self-evident.

(d) In situ double nerve-muscle preparation: Using electronic stimulators, a more effective stimulus is delivered when one electrode is placed under the sciatic nerve and a spade terminal attached to the foot of the undissected leg. A frog is pithed, a piece of thin string, about ten inches long, is tied to each hand and foot, and the animal is secured to the dissecting board by tying these strings to a pin, those from the feet passing below the board. The skin is removed from one leg, and a 1½-inch pin driven through the lower end of the femur. The iliofibular and triceps femoris muscles are separated to expose the sciatic nerve, which is then carefully parted from the sciatic artery. A short piece of thin string, soaked in frog Ringer, is passed under the sciatic nerve, above the artery, and tied firmly at the front of the thigh, so that it immobilises the thigh muscles and occludes the blood supply to the leg. The two selected muscles are prepared as above, (1c). A further piece of string is tied around the body of the frog below the armpits. The frog is then transferred to the bath and tied in position, so that the stump of the tibiofibula lies along the midline of the bath. The strings from the hands and remaining foot, and round the body, are tied to the stand to prevent movements of the body from affecting the trace. The pin and knee are secured as above and the muscles attached to the recording device (double myograph or transducers, as the case may be).

(e) The partly decerebrate preparation: Where convulsants are used as a substitute for electrical stimulation, or to study their actions, the animal cannot be pithed. To effect a partial decerebration, a seeker is pushed through the roof of the skull in the mid-dorsal line, immediately posterior to the orbits, and its point turned forward and rotated, to destroy the whole of the brain anterior to the origin of the fourth cranial nerves. Destruction of the medulla oblongata is found to prevent continuous convulsions, including those induced by strychnine. The preparation is exactly as described for the in situ on (1d) above.

(f) The whole animal preparation: A limited number of experiments were performed on intact preparations to confirm that partial decerebration, as described above (1e) did not significantly alter the pattern of convulsions. It is virtually certain that an animal undergoing convulsions will be unconscious.

II STIMULATION

(a) The du Bois Reymond inductorium: The standard induction coil. Used to deliver maximal break shocks, either singly or repetitively.

(b) Simple neon-discharge stimulator: The circuit diagram for the neon-discharge stimulator is illustrated in Fig. 2. For repetitive stimulation, a 700-ohm Omron relay was inserted in the output and operated by a press-button switch in conjunction with a minute time clock.

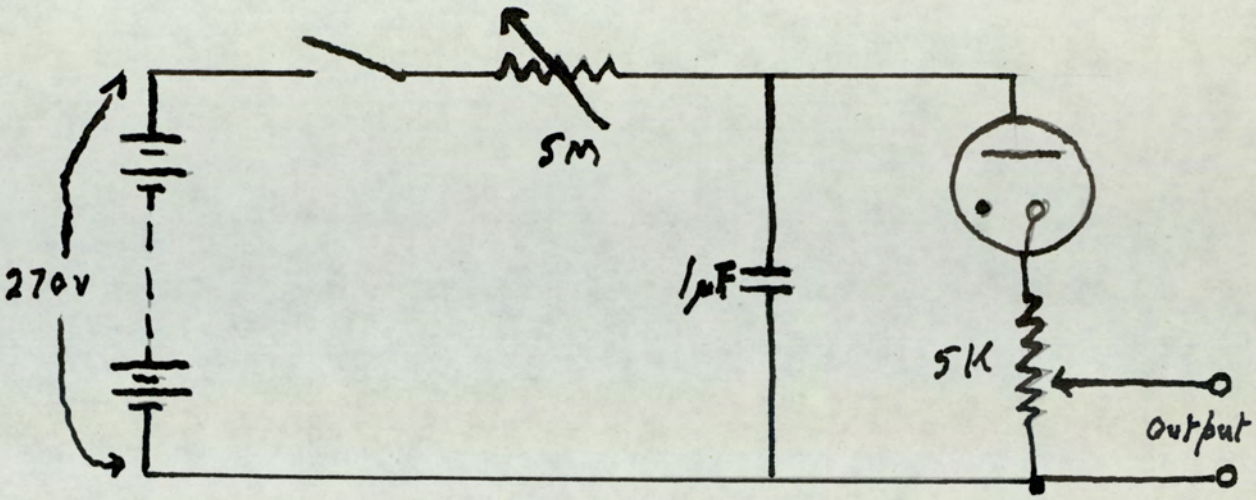


Fig. 2: Circuit diagram of simple neon-discharge stimulator.

(c) Palmer's H45 electronic square-wave stimulator.

(d) Subcutaneous injections of convulsants, sometimes preceded by anticonvulsants. Injections (in 0.1 to 1ml. frog Ringer) made into the dorsal lymph sac, above the skull. Steriseal 1ml. sterile disposable syringes, luer fitting were used.

III MYOGRAPHS

(a) Simple myograph for use with the Keith Lucas muscle bath. Standard equipment, supplied by Palmer's.

(b) Original double myograph for use with Keith Lucas muscle bath: The original double myograph, made to specification by Palmer's is illustrated in Plate 14. It carried two straws with tangential writing points for recording on smoked kymograph paper.

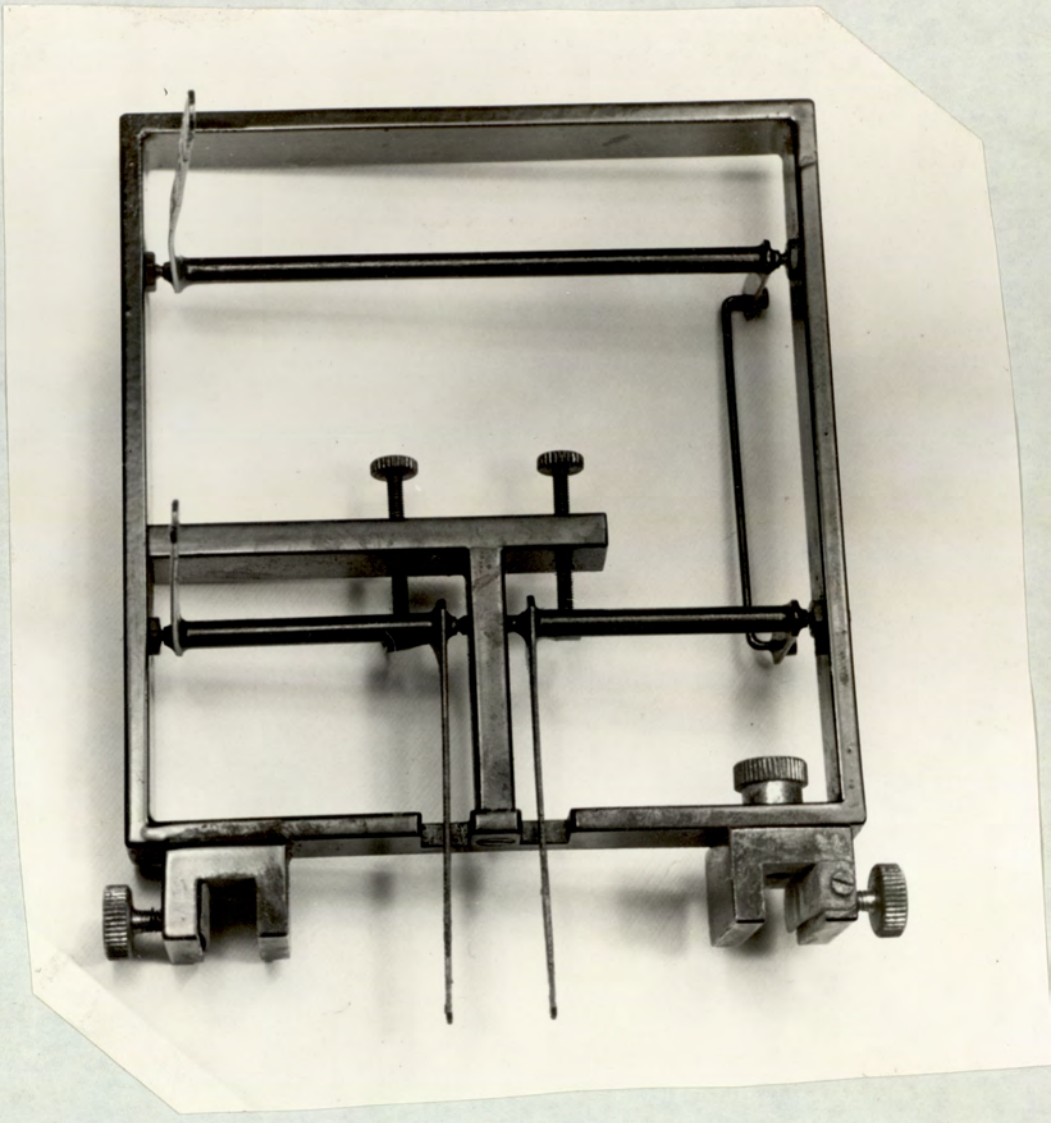


Plate 14: The original double myograph.

(c) Modified double myograph for use with Keith Lucas muscle bath: In view of the difficulty of using a tangential writing lever in conjunction with a cylindrical drum, the original double myograph was modified to carry frontal writing levers. The frame consists of an upright arm, 12.3cm. x 1.2cm. x 0.2cm., drilled by two holes, each 6mm. in diameter, to carry rods, which bear the pivot housings for the frontal writing levers, centred at 0.6cm. and 9.8cm. from the top, and a horizontal bar (Fig. 3). The horizontal arm, 5.8cm. x 1.2cm. x 0.2cm., has the front cut away, so that a tongue, 3.3cm. x 0.7cm. x 0.2cm., projects from the base (Fig. 4).

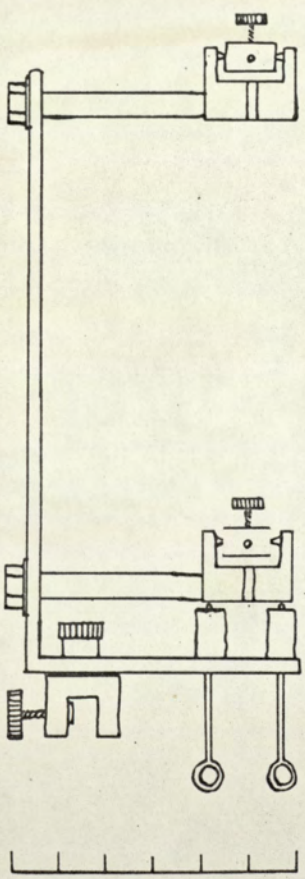


Fig. 3. Double myograph, Front elevation. Scale 1 cm.

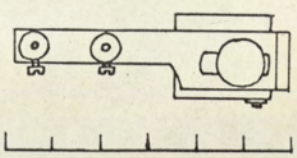


Fig. 4. Horizontal arm of the frame, from above. Scale, 1cm.

A slot, 1.4cm. x 0.4cm., is cut in the base to carry the bracket which fits over the side of the bath. The tongue is drilled by two holes, each of 2mm. diameter, centred at 4 mm. and 2cm. from the free end, each of which carries a collar, 1cm. high, 7mm. external, and 2mm. internal diameter, fitted with a grub screw for adjusting and securing an eyelet loop.

Standard frontal writing levers are modified by cutting down the rods which bear the pivot housings to a length of 4.2cm. and turning the ends for 8mm., so that they can be bolted to the upright of the frame to carry the levers over the centre of the bath (Fig. 5). Shafts 39cm. long were used as levers, to which the frontal writing points were attached. The levers were counterbalanced by collars. A wire hook introduced between the collars facilitates attachment of threads from the muscles.

The bath bracket, a saddle, 1.9cm. x 1.6cm. x 1.3cm., is cut out to form a groove, 1.9cm. x. 0.8cm. x 0.8cm., which fits over the side of the bath. A thin brass strip, 2.4cm. x 5mm. x 0.05mm., is attached to the front, and bent to line the outer edge of the groove. The bracket carries a screw above, which is passed through the slot in the base and secured by a knurled knob. The saddle is secured to the bath, after adjustment, by a screw with a knurled head, the end of which bears on the brass strip.

The eyelet loop should be of metal resistant to saline, and have an external diameter of 6mm., an internal diameter of 4mm., and a stem 3.3cm. long. A pair of self-aligning pulleys may be fixed to the bath to give the threads from the muscle tendons an almost vertical pull.

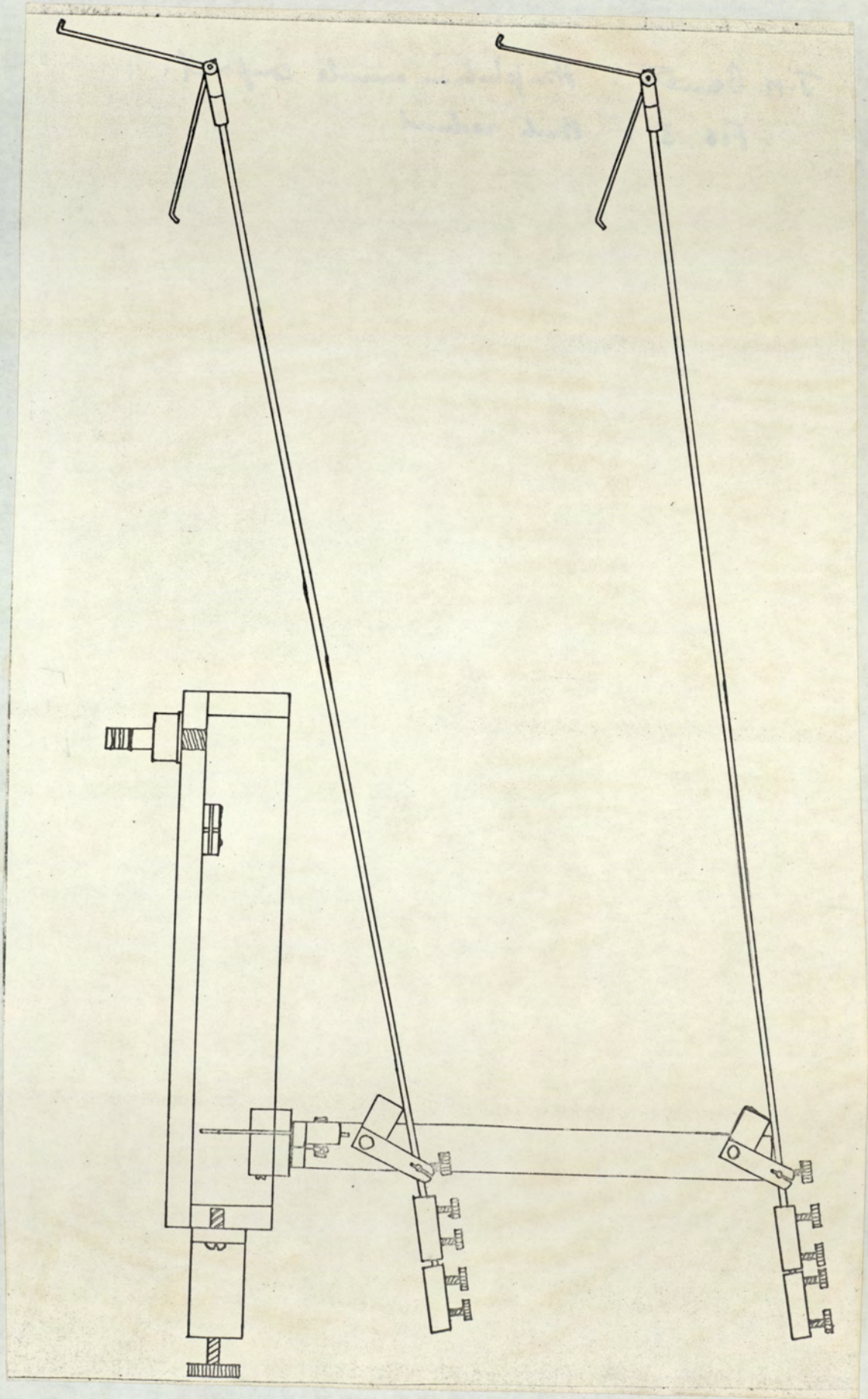


Fig. 5: Double myograph. Side elevation.

(d) The use of transducers: This has been previously described (Bennett, 1970). The threads were attached, via self-aligning pulleys, to Devices UF1 220g. dynamometers and changes in tension recorded by a Devices M2 electronic recorder. The initial tensions were independently zeroed by rack and pinion mounting of the strain gauges. The calibration was effected directly by suspending known weights from the arms of the transducers and marking the point reached by the recording pen at each tension.

IV RINGER SOLUTION

The composition of frog Ringer used in the bath was: NaCl, 0.6%; KCl, 0.019%; CaCl, 0.024%; $NaHCO_3$, 0.01%, glucose. 0.1%.

SIMPLE ISOMETRIC MUSCLE TWITCH

Peachey and Huxley (1962) found that slow fibres in the tonus bundle of iliofibularis of the frog failed to respond mechanically to single stimuli, but responded to adequate stimuli applied at rates of 10 per second, or greater. A comparison between simple isometric muscle twitches of tibialis anticus and peronaeus longus may, thus, be taken to be a comparison between the twitch fibres in the two muscles.

METHOD: The in situ double nerve-muscle preparation (p. 39, d) was used, the sciatic nerve being stimulated by a Palmer's electronic square-wave stimulator and the recording was by transducers (p. 46, d). The stimulus was 7v x 0·8msec.

RESULTS:

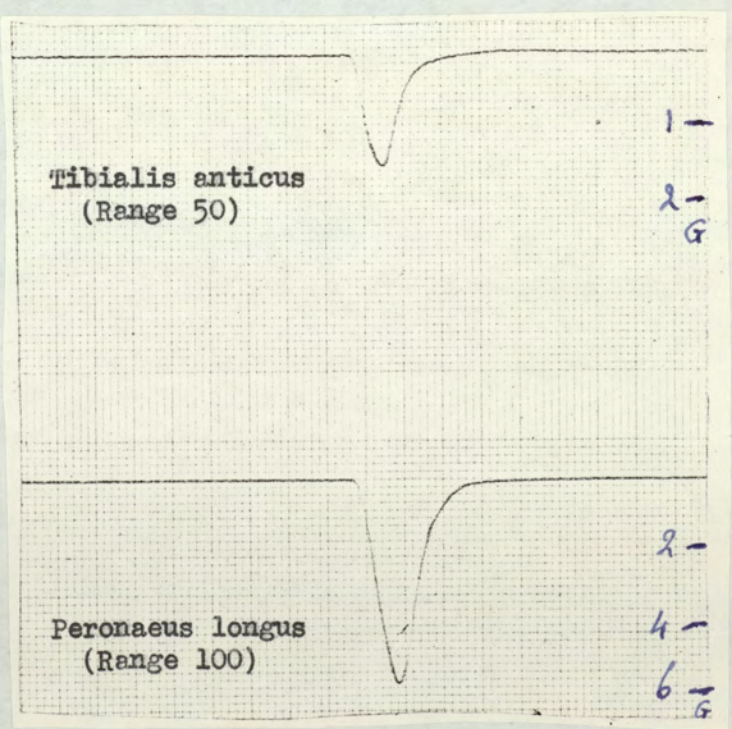


Fig. 6. Double myogram. Frog (Rana temporaria). Left leg. Drum-speed, 100 mm/sec.

	Latent period	Shortening		Total twitch		Twitch tension	
	Ext - Fl	Fl	Ext	Fl	Ext	Fl	Ext
	μsec.	μsec	μsec	μsec	μsec	g.wt.	g.wt.
R	6.0	35.0	40.0	190	200	0.9	1.2
L	5.0	45.0	47.5	105	155	0.9	4.2
L	2.5	40.0	60.0	129	171	1.3	5.5
R	0.0	60.0	55.0	160	155	1.8	2.5
L	0.0	42.5	55.0	140	150	0.6	1.2
R	0.0	49.0	49.0	130	135	0.8	1.0
L	2.5	55.0	40.0	160	120	0.6	1.0
R	0.0	52.5	65.0	120	180	0.7	1.0
Mean	2.0	47.38	51.44	141.75	158.25	0.95	2.20
SEM ±	0.86	2.94	3.18	9.56	8.94	0.15	0.62
P <		0.20		0.15		0.05	

Table 5. Comparison of parameters of simple isometric muscle twitch between tibialis anticus (Fl) and peronaeus longus (Ext) in the frog (Rana temporaria). R = Right leg; L = Left leg. In situ preparation. Latent period expressed as difference, positive indicating that of the flexor has the shorter duration. Total twitch time excludes the latent period.

A typical tracing is illustrated in Fig. 6, and the results of 8 experiments tabulated in Table 5.

DISCUSSION: The inertia of the system, the relatively slow drum-speed, and dependence on exact alignment of the drum paper may be responsible for the poor significance of the measurements involving time. Where

a difference in latency can be detected, that of the flexor is shorter than that of the extensor. The time of shortening and the total twitch time, excluding the latent period, are, on average, less than those of the extensor. The twitch tension produced by tibialis anticus is significantly less than that by peronaeus longus. To this extent, there is some indication of adaptation to the respective functions of rapid withdrawal and powerful thrust.

FEATURES OF THE TETANUS

Whitney (1958) precluded tetanic activation of individual fibres of a muscle as a feature of normal muscular activity. Clare and Bishop (1957), however, attributed the increased amplitude of response under strychnine to an increase in the number of units fired in each recruiting wave. Since the extra elements might be fired nearly synchronously with those responding immediately, or after a variable delay, the production of a tetanus by convulsants is by no means ruled out. Wells (1965) found that maximal tension in the soleus of young rats declined 50% in 143 seconds, compared to 11 seconds in the anterior tibial muscle and Aidley (1971) includes fusion frequency among the characteristics of muscle. It therefore seemed appropriate to investigate the features of a tetanus in the muscles under review.

METHODS. After recording the simple isotonic twitches used for obtaining the data for Table 5, the frequency of stimulation was varied, its nature being unaltered (7v x 0.8msec. square-wave).

RESULTS. The results are illustrated in Fig. 7 and tabulated in Table 6.

Legend to Fig. 7, p. 51: Genesis of a tetanus in tibialis anticus (Fl) and peronaeus longus (Ext) in the frog (Rana temporaria). Rate of stimulation (7v x 0.8msec. square-wave) per second. Tension = g.wt. Devices UFl 220g. dynamometers with Devices M2 recorder.

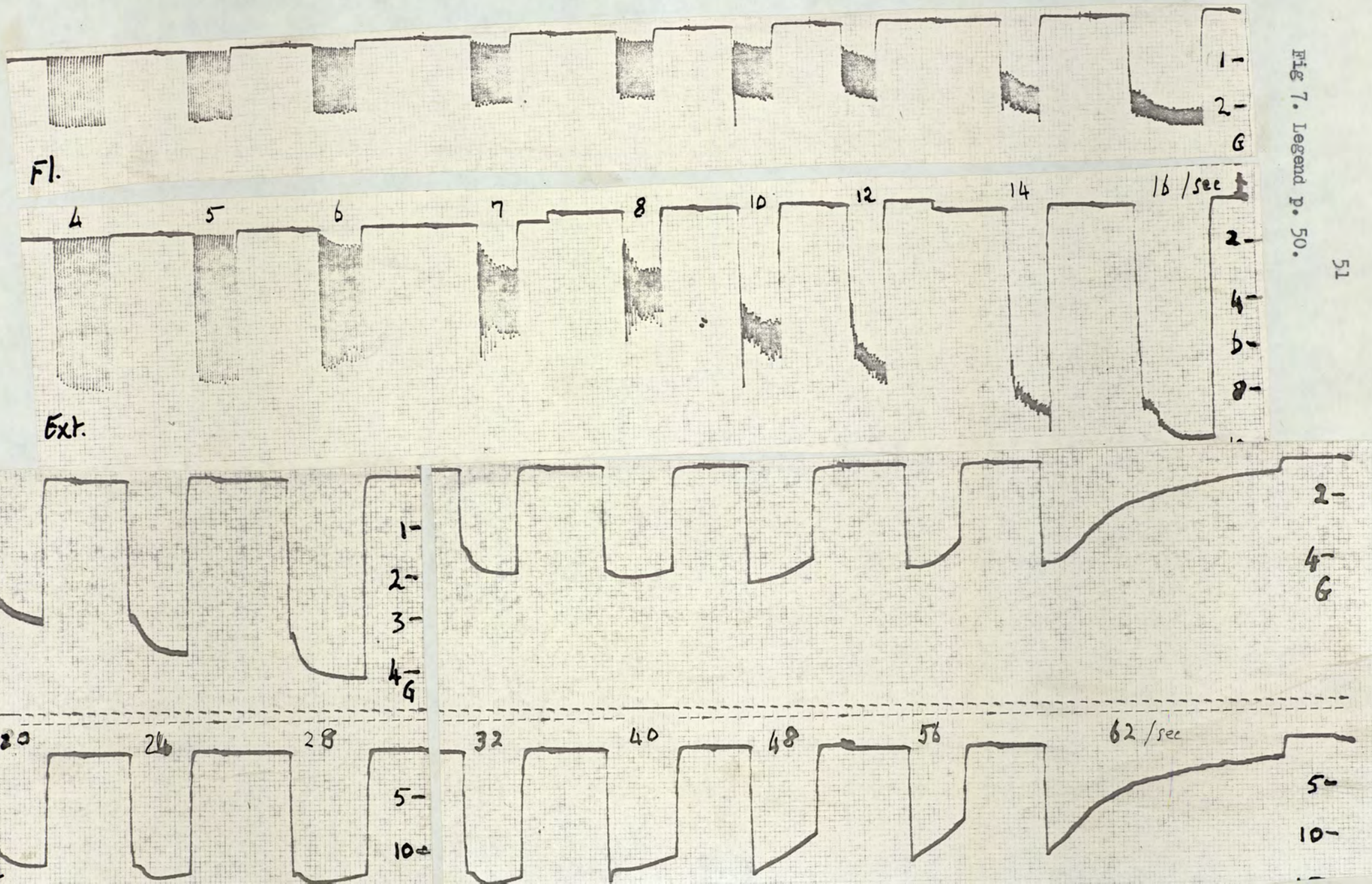


Fig 7. Legend p. 50.

L/R	Fusion frequency		Maximum tetanus tension		Time to 50% decline in tension	
	Stimuli/sec.		g.wt.		sec.	
	Fl	Ext	Fl	Ext	Fl	Ext
L	28	20	4.1	11.0	6	7
L	20	16	5.8	14.0	36	18
R	20	14	8.1	2.5	16	-
R	14	24	11.5	4.5	10	6
L	32	24	5.0	10.1	17	16
R	20	24	10.2	9.8	5	8
L	24	24	1.9	4.8	13	11
L	20	20	21.0	6.3	14	4
Mean	22.25	20.75	8,45	7.88	14.75	10.00
SEM ±	1.98	1.41	2.12	1.39	3.42	1.99
P <	0.30		0.49		0.15	

Table 6. Comparison of features of a tetanus between tibialis anticus (Fl) and peronaeus longus (Ext) in the frog (Rana temporaria). L = left leg; R = right leg

DISCUSSION. The results are of poor statistical significance, but indicate that the flexor has the slightly higher fusion frequency and maintains a tetanus better than the extensor. The latter is likely to be due to the slow fibres present in tibialis anticus but absent from peronaeus longus. From experiments with convulsants described later, the extensor seems to be capable of developing the greater tension, but the fact that slow fibres are activated at frequencies in excess of ten per second may account for a smaller difference in tetanic than in twitch tension between the muscles.

THE ACTION OF DRUGS AT THE NEUROMUSCULAR JUNCTION

1. STRYCHNINE

A. EFFECT ON SIMPLE ISOTONIC TWITCH

The first investigation in the current project concerned the effect of strychnine on the simple muscle twitch of the gastrocnemius muscle of the frog (Rana temporaria).

METHOD: The sciatic-gastrocnemius preparation (p. 37, Ia) was used in a Keith Lucas muscle bath, containing 150ml. frog Ringer (p. 46, IV). The sciatic nerve was stimulated by single maximal break shocks from a du Bois Reymond inductorium (p. 40, IIa). A simple muscle twitch was recorded on a smoked kymograph drum by a simple tangental isotonic lever; a known dose of strychnine, as hydrochloride, was added to the Ringer in the bath and a second twitch was recorded. The Ringer was changed, a control twitch recorded, another dose of strychnine added, a further twitch recorded, and the Ringer changed. This sequence was repeated for varying doses of strychnine between 1µg and 5mg. A time base was recorded by tuning fork giving 100 vibrations per second. The latent periods were measured by travelling microscope, and the heights of twitch directly. Twitches were recorded separately, except on one occasion where they were superimposed (Fig. 9).

RESULTS: The results are illustrated in Figs. 8 and 9, and tabulated in Table 7.

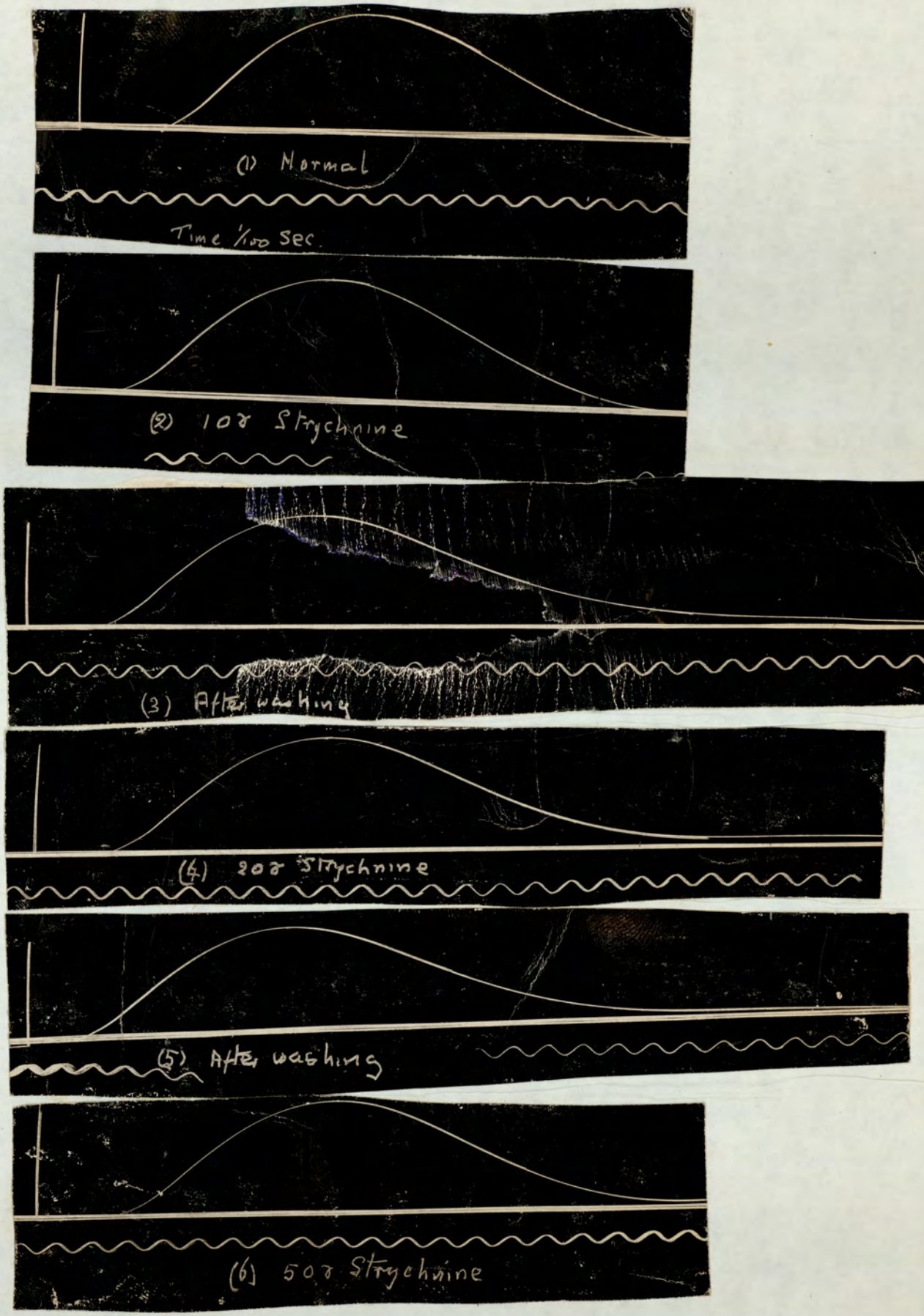


Fig. 8. Effect of strychnine on isotonic twitch. Gastrocnemius of frog (Rana temporaria). Time = 1/100 sec.

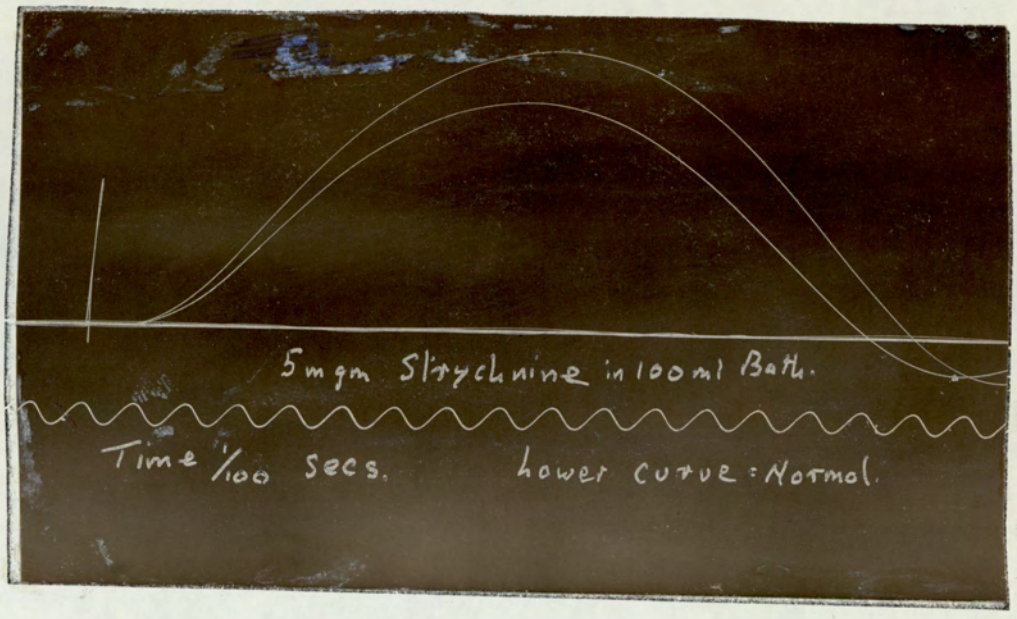


Fig. 9. Effect of strychnine on isotonic twitch, responses superimposed. Time, 1/100th. second.

Dose in 150 ml.		Change in latent period mm on trace	Increase in height mm on trace
0	(1)	+ 0.20	0
1 µg	(1)	+ 0.70	0
2 µg	(1)	+ 0.12	0
5 µg	(1)	- 0.01	1.4
10 µg	(3)	- 0.24	1.9
20 µg	(3)	- 0.10	0.8
50 µg	(2)	+ 0.16	0.7
100 µg	(1)	+ 0.10	1.4
5 mg	(4)	+ 0.06	0.1

Table 7. Mean change in latent period and mean increase in height of contraction of gastrocnemius muscle of frog (Rana temporaria) exposed to varying doses of strychnine in 150ml. frog Ringer. Number of determinations in brackets.

Considering the means of all data in Table 7 for doses of strychnine in excess of 5 µg. per 150 ml. frog Ringer, there is a shortening of the latent period of 0.06 mm. (= 0.09 msec.) and an increase in height of contraction of 0.91 mm. (= 3.6%).

B. EFFECT ON TETANUS

The effect on the simple muscle twitch not proving readily demonstrable with the equipment available to students, it was decided to investigate any action on a tetanus. Originally, this was applied to the gastrocnemius of the frog but, within a month, the tibialis anticus was selected to represent an antagonist of that muscle.

METHODS: (i) Separate recording of responses: A sciatic-gastrocnemius preparation (p.37, Ia) was set up and the nerve stimulated by a du Bois Reymond inductorium (p. 40, IIa) with maximal break shocks from the vibrator for the first five seconds of every twenty-second period, the responses of the muscle being recorded by a simple tangential writing lever recording on a smoked kymograph paper. A sciatic-tibialis anticus preparation (p. 38, Ib) was made from the opposite leg of the frog, and the experiment repeated. The whole exercise was then repeated, adding varying doses of strychnine, as hydrochloride, to the Ringer (p. 46, IV) in the bath.

(ii) Double myograph: An isolated double nerve-muscle preparation (p. 38, Ic) incorporating gastrocnemius and tibialis anticus was set up. The experimental procedure of Method (i), with respect to stimulation and recording, was used.

RESULTS: Results from Method (i) are illustrated in Fig. 10, and from Method (ii) in Fig. 11.

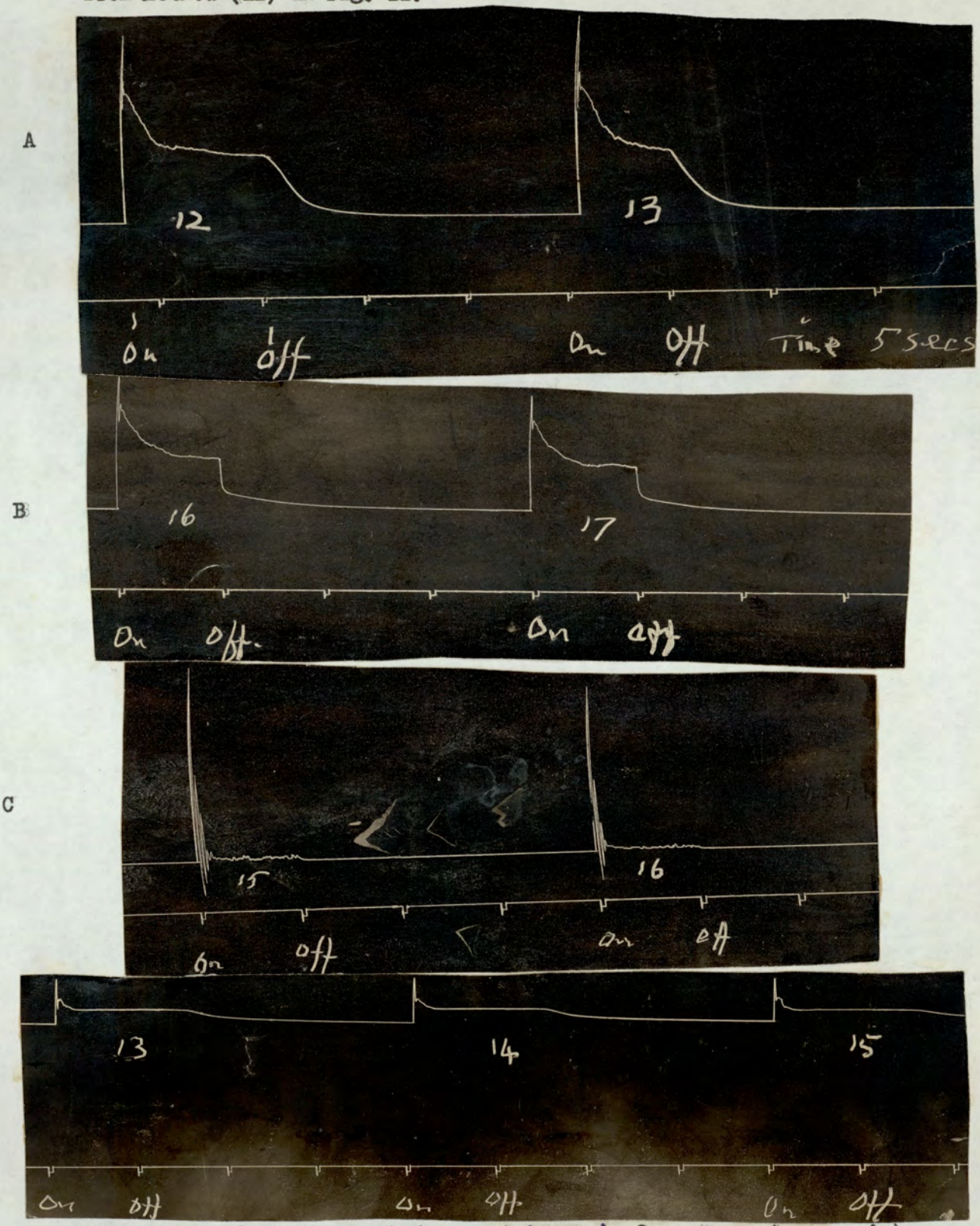


Fig. 10. Effect of strychnine on tetanus in frog (Rana temporaria). Preparation stimulated first 5 sec. of each 20-sec. period @ 100 Hz. A = gastrocnemius control; B = tibialis anticus control; C = gastrocnemius exposed to strychnine, 50 µg/ml; D = tibialis anticus exposed to strychnine, 50 µg/ml. Time = 5 sec.

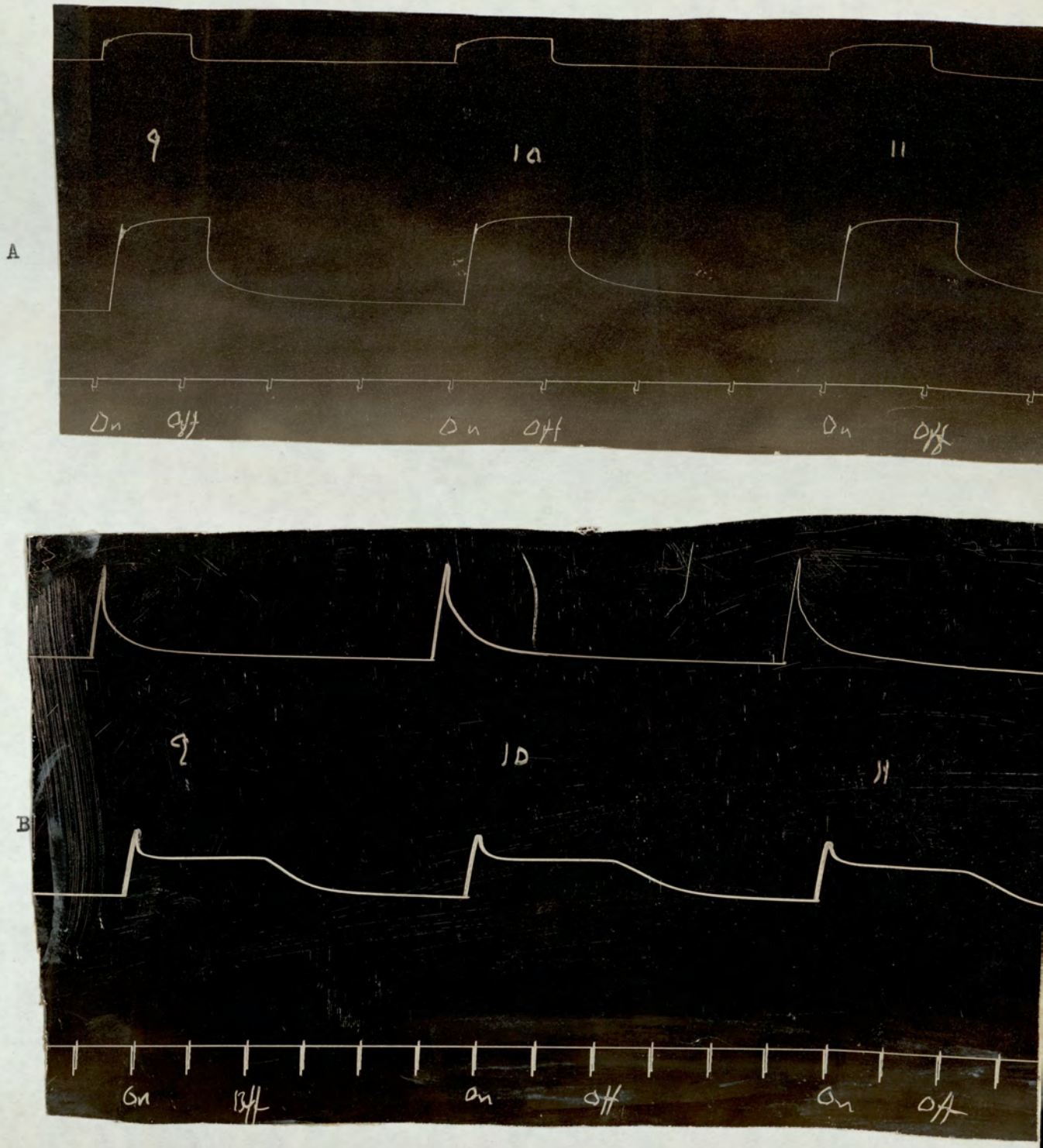


Fig. 11. Effect of strychnine on tetanus in frog (Rana temporaria). Double myographs: A = Control; tibialis anticus above; gastrocnemius, below; sciatic nerve stimulated first 5 sec. in each 20-sec. period. B = Exposed to strychnine (50 ug/ml.), gastrocnemius, above; tibialis anticus, below; sciatic nerve stimulated first 10 sec. in each 30-sec. period. Time = 5 sec.

It is found, using either Method (i) or Method (ii), that the sequence of stimulating either muscle for the first five seconds in each twenty-second period (or for the first ten seconds of each thirty-second period) produces similar control tetani in the two muscles, without undue evidence of fatigue. The tetanus plateau is maintained throughout the period of stimulation and the muscles relax rapidly on conclusion of stimulation. When exposed to strychnine (5mg. in 100ml. frog Ringer in the bath), the gastrocnemius relaxes almost completely during the stimulation period, but tibialis anticus maintains its tension well beyond the cessation of stimulation.

Using a single lever, it was found that suitable results were obtained by isolating a double nerve-muscle preparation and attaching one of the two muscles to the lever and conducting the experiment (Method (i)), then detaching this muscle and attaching the other and repeating the experiment. In the controls, the second muscle used showed little evidence of fatigue due to passive contractions during stimulation; in the tests, the second muscle showed little evidence of penetration by strychnine during its passive contractions. Thus, if a double myograph is not available, results can be obtained using the one leg of a frog, which may be preferable to employing opposite legs on physiological, as well as economic, grounds.

Note: The results were subsequently confirmed using the modified double myograph (p. 43, IIIc) in conjunction with the neon-discharge stimulator (p. 40, IIb).

C. EFFECT ON SUCCESSIVE ISOMETRIC TWITCHES

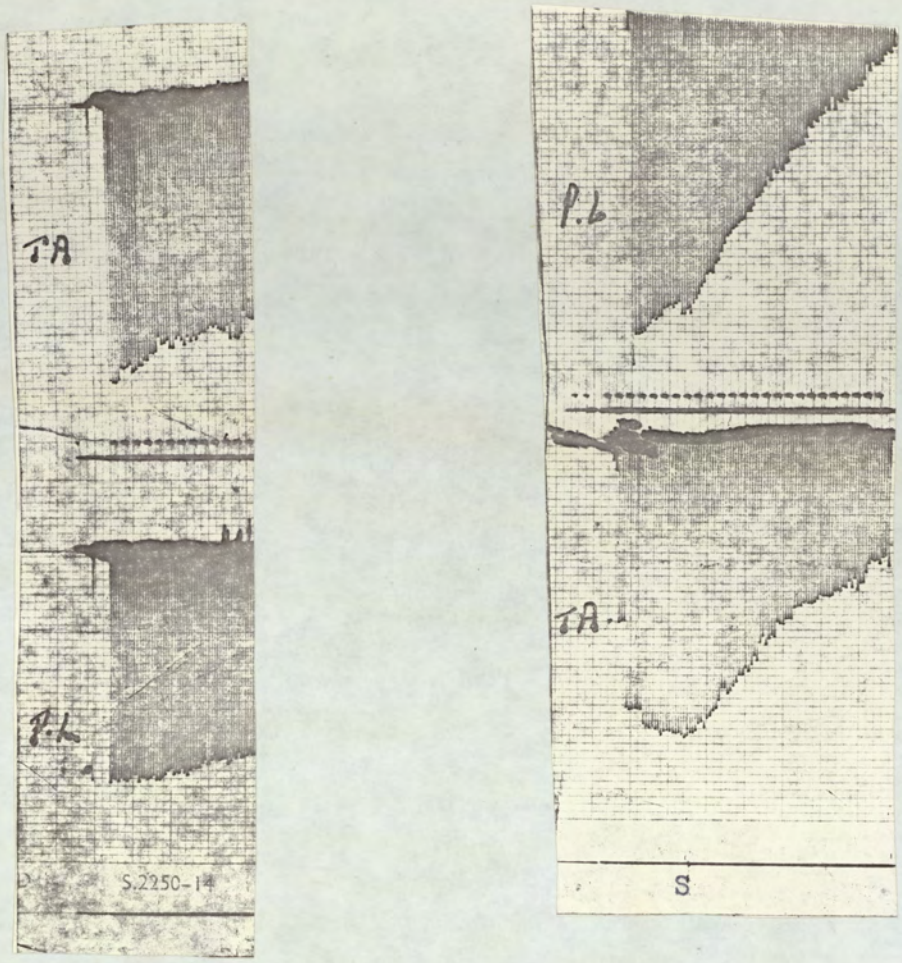
The effects of neuromuscular blocking agents are frequently investigated by applying a series of single maximal electrical stimuli indirectly to a nerve-muscle preparation at a rate which does not induce significant fatigue (e.g. Veley and Walker, 1909; Jewell and Zaimis, 1954). Peronaeus longus was selected as the antagonist of tibialis anticus, since, unlike gastrocnemius, it contains no slow fibres.

METHOD: The in situ double nerve-muscle preparation (p. 39, Id) was used, incorporating tibialis anticus and peronaeus longus of the frog (Rana temporaria). The sciatic nerve was stimulated once every 16 seconds by a square-wave of 6v. x 40μsec. (p. 41, IIc). Responses of the muscles were recorded by an electronic recorder in conjunction with strain gauges (p. 46, IIId). The sensitivity of the channels was adjusted so that trial responses of the two muscles were about equal. Strychnine (4 - 6mg.), alone and followed by suxethonium (Brevidil E, 0·8mg.), was added to 150ml. frog Ringer (p. 46, IV) in the muscle bath. Controls, without drug, and with suxethonium alone, were recorded.

RESULTS: The results are illustrated in Figs. 12 and 13.

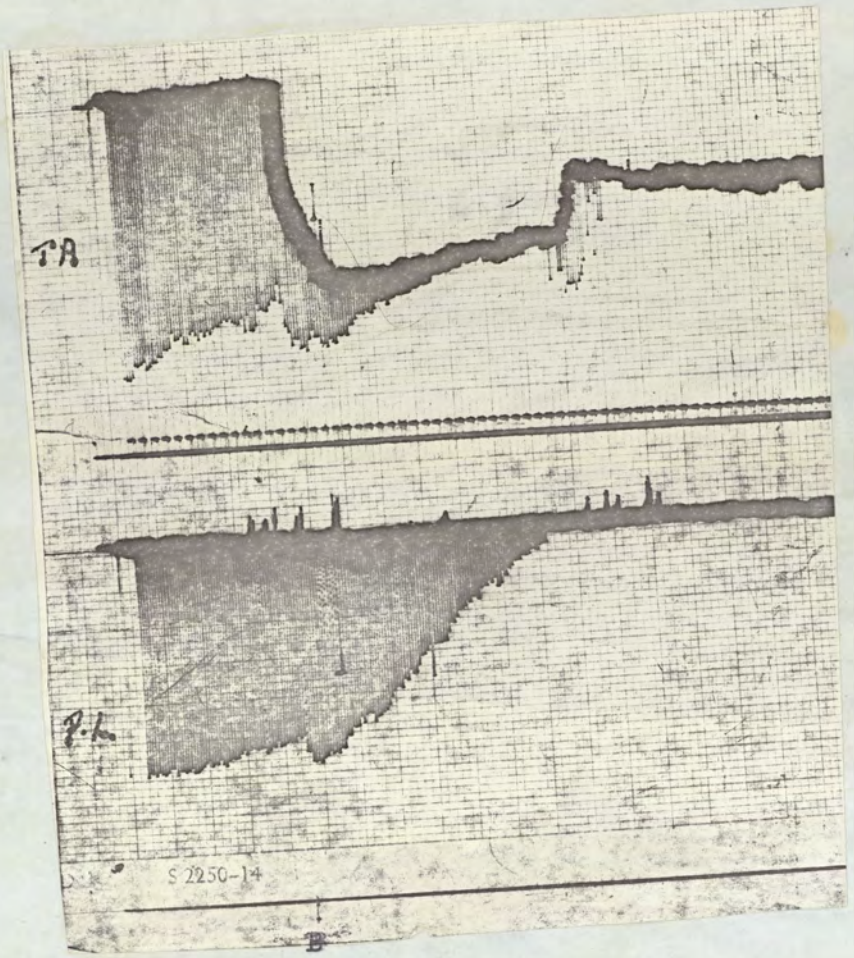
Strychnine was found to have a curare-like action on both muscles. The tensions developed by the muscles was progressively reduced, with no initial potentiation.

Suxethonium caused an initial potentiation of twitch fibres which was followed by a progressive reduction in tension, together with a contracture of the slow fibres in tibialis anticus. Pretreatment with strychnine largely prevented the contracture.

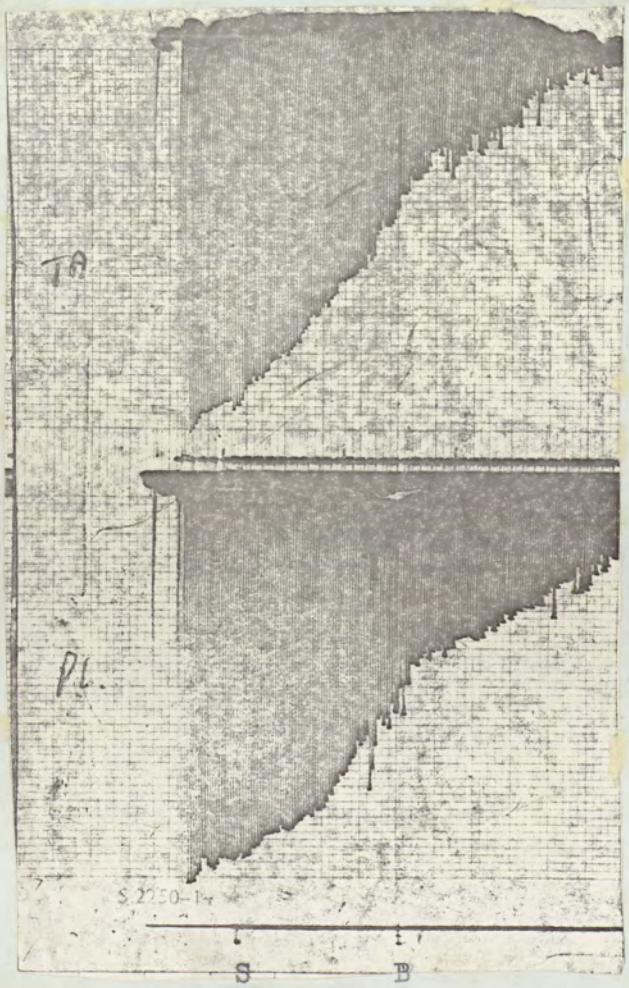


(a) Control. (b) Test.

Fig. 12. Curare-like action of strychnine on muscles of the frog (Rana temporaria). Double myogram of tibialis anticus (T.A.) and peronaeus longus (P.L.). Sciatic nerve stimulated by single square-waves (6v. x 40μsec.) every 16 seconds. Time, 1 minute. (a) Control, (b) Test. At S, 6mg. strychnine, as hydrochloride, added to 150ml. frog Ringer in muscle bath.



(a) Action of suxethonium alone



(b) Test.

Fig. 13. Effect of pretreatment with strychnine on response to suxethonium (Brevidil E) in muscles of the frog (Rana temporaria). Double myogram of tibialis anticus (T.A.) and peronaeus longus (P.L.). Sciatic nerve stimulated by single square-waves (6v. x 40μsec.) every 16 seconds. Time, 1 minute.
(a) Control, 0.8mg. suxethonium added to 150ml. frog Ringer at B.
(b) Test. At S, 4mg. strychnine, and at B, 0.8mg. suxethonium added.

The twitch fibres of tibialis anticus are less sensitive to strychnine than those of peronaeus longus (Table 8):

Dose	Tibialis anticus	Peronaeus longus
1mg.	69.3% in 20 min.	80.6% in 20 min.
1mg.	100% in 19 min.	100% in 17 min.
2 mg.	100% in 24 min.	100% in 20 min.
10 mg.	100% in 18 min.	100% in 10 min.

Table 8. Comparison of sensitivities of twitch fibres in tibialis anticus and fibres of peronaeus longus to strychnine in the frog (Rana temporaria). Dose in 150ml. frog Ringer. % reduction of height of responses to successive single square-waves (6v. x 40μsec.) in stated time.

2. SUXETHONIUM (BREVIDIL E).

Suxethonium (Brevidil E) was selected to exemplify neuromuscular blocking agents of the depolarizing type.

METHOD: The method was the same as that used for investigating the effect of strychnine on successive isometric twitches (p. 60).

RESULTS: The results are illustrated in Fig. 13 (a). Slow fibres do not respond to individual stimuli given at a rate of one every 16 seconds. The first effect of adding suxethonium to the frog Ringer in the bath is a potentiation of the responses of the twitch fibres of tibialis anticus and of peronaeus longus. A contracture of the slow fibres of tibialis anticus rapidly ensues and this, when maximal, may mask the

responses of the twitch fibres of that muscle. Finally, the responses of the twitch fibres are reduced, eventually being abolished by high doses.

Dose	Maximum block achieved Tibialis anticus	Maximum block recorded Peronaeus longus
mg.	Slow fibres	Twitch fibres
0.1	33% in 6 min.	3% in 10 min.
0.2	16% in 3 min.	40% in 20 min.
0.3	40% in 9 min.	20% in 18 min.
0.4	32% in 8 min.	58% in 20 min.
0.5	46% in 6 min.	16% in 20 min.
0.6	45% in 5 min.	55% in 9 min.
0.8	72% in 4 min.	100% in 25 min.

Table 9. Comparison of sensitivity of slow fibres in tibialis anticus and twitch fibres of peronaeus longus to suxethonium in the frog (Rana temporaria). For slow fibres, the height of contracture is expressed as a percentage of the height of contraction of twitch fibres prior to application of the drug; for twitch fibres, the block is expressed as a percentage of the height of contraction prior to application of the drug. Slow fibres achieved their maximum contracture during the recording, twitch fibres did not appear to reach their maximum blockade during this time, except at the highest dose.

The greater sensitivity of the slow fibres of tibialis anticus compared with that of the twitch fibres of peronaeus longus is most apparent in the speed of onset of action. The relative sensitivities

of the twitch fibres in tibialis anticus and of peronaeus longus cannot readily be assessed because, as has been stated, the contracture masks their responses. The initial potentiation of the twitch fibres of tibialis anticus tends to be greater than that of those of peronaeus longus, indicating that the former may be the more sensitive.

DISCUSSION: Langley (1905) demonstrated the antagonism between nicotine and curare on the contractures of avian muscle and adduced evidence that neuromuscular blocking agents, including nicotine, curare, and strychnine act upon a receptor substance in the cells. In 1906, he showed that the portion of frog sartorius which contained nerve-endings was more sensitive to such agents than the ends of the muscle. In 1907, by applying solutions of nicotine to the surface of skeletal muscle cells with a fine sable brush, he found that the flexors of the fore-limbs (pectoralis abdominis, coracoradialis, and flexor carpi radialis) of the pithed or decerebrate frog gave weak, brief twitches followed by a marked contracture, whereas the muscles of the hind-limbs (of which only sartorius is specified) were less sensitive and the duration of the tonic contraction was less.

The classification of muscles of the frog by Sommerkamp (1928), which has been mentioned earlier (p. 3), depended in the first instance on the results of applying acetylcholine to individual muscles. He compared the physiological and pharmacological properties of the tonus bundle of iliofibularis with those of the non-tonic part of that muscle. Acetylcholine, nicotine, sodium thiocyanate, and low doses of potassium caused contractures of the tonus bundle, whereas quinine, caffeine, and high doses of potassium evoked contractures in both parts. A classification of limb muscles of the frog, based on the work of

Langley (1905, 1906, 1907), Sommerkamp (1928), Gray (1958) and the present work is given in Table 10:

Purely tetanic muscles	Muscles containing twitch and slow fibres randomly distributed	Muscles containing one or two tonus bundles
Sartorius	Pectoralis abdominis	Iliofibularis
Semimembranosus	Coracoradialis	Semitendinosus
Gracilis minor	Flexor carpi radialis	Gastrocnemius
Peronaeus longus	Adductor magnus	
	Ext. long. dig. IV	
	Tibialis anticus	

Table 10. Classification of limb muscles of the frog

In general, this classification shows that extensors are purely tetanic, flexors contain 'twitch' and 'slow' fibres randomly distributed, and muscles which act on two joints have tonus bundles.

Jewell and Zaimis (1954) differentiated between red and white muscles in the cat by the use of neuromuscular blocking agents. They employed the homogenous red soleus and the heterogenous tibialis anterior, in which white fibres predominate. In comparing their results with the present ones, the analogies must be clearly appreciated (Table 11):

Cat		Frog
Red fibres of soleus	=	Twitch fibres of peronaeus longus
White fibres of tibialis ant.	=	Twitch fibres of tibialis anticus
Red fibres of tibialis ant.	=	Slow fibres of tibialis anticus

Cat		Frog gastrocnemius
Red fibres of soleus	=	Twitch fibres of non-tonic part
White fibres of tibialis ant.	=	Twitch fibres of tonus bundle
Red fibres of tibialis ant.	=	Slow fibres of tonus bundle.

Table 11. Analogies of fibres in mammalian and amphibian muscle.

Difficulties which have confronted previous workers have arisen from the assumptions that, in the frog, twitch fibres are indistinguishable and that, in the mammal, red fibres are indistinguishable. A further difficulty is that slow fibres in the frog receive multiple innervation and undergo contracture when depolarizing agents are applied, whereas the red fibres of tibialis anterior in the cat are focally innervated and have not been shown to undergo contracture in these conditions. Any adaptation of the mammalian flexor to the function of maintaining the withdrawal of a limb is presumably brought about by a quite different mechanism than in the frog.

The results of Jewell and Zaimis (1954) as attributed by them to red and white muscle are summarized in Table 12:

	White fibres of tibialis ant.	Red fibres of soleus
Speed of contraction	Fast	Slow
Fusion frequency	High	Low
Sensitivity to tubocurarine	Lower	Higher
Sensitivity to decamethonium	Higher	Lower
Mode of action of decamethonium	Depolarizing	Dual
Successive doses of decamethonium	Sensitization	Tachyphylaxis
Tetanus during decamethonium block	Maintained	Not maintained
Initial tetanus tension in block	Low	High
Effect of tetanus on block	Not antagonized	Antagonized
Effect of neostigmine on block	Not antagonized	Antagonized
Effect of tubocurarine on block	Antagonized	Deepened

Table 12. Comparison between white fibres of tibialis anterior and red fibres of soleus of cat. Data from Jewell and Zaimis (1954).

The only properties the authors attributed to red fibres in the tibialis anticus were a slight antagonism which neostigmine was found to produce on a deep decamethonium block; a short-lasting deepening of the block which preceded the tubocurarine antagonism of decamethonium, and their ability to demonstrate tachyphylaxis in this muscle under certain circumstances. The results of the present work indicate that the analogies given in Table 11 are consistent with the first three lines of Table 12, comparable results for the remainder not being available.

McPhedran, Wuerker and Henneman (1965) confirmed that the soleus of the cat was an homogenous muscle. The same authors (Wuerker, McPhedran and Henneman, 1965) described three types of motor unit in the gastrocnemius of the cat: the majority of units gave twitches and had a high fusion frequency, these could correspond to the white fibres

of tibialis anterior in the cat and the twitch fibres of the tonus bundle of gastrocnemius in the frog; other units gave twitches but had a low fusion frequency, these could correspond to the red fibres of soleus in the cat and the twitch fibres of the purely tetanic part of gastrocnemius in the frog, while the third group did not give twitches but were capable of developing a tetanus, these could correspond to the red fibres of tibialis anterior in the cat and the slow fibres in the tonus bundle of gastrocnemius in the frog. The proportions of the various classes of motor unit found in the gastrocnemius of the cat differ markedly from those of their probable analogues in the frog. It may be that the ventral roots employed for stimulation served the equivalent of the tonus bundle predominantly.

Most authors investigating neuromuscular transmission and its modification by drugs treat skeletal muscles as uniform. Thus, Dale, Feldberg and Vogt (1936) showed that stimulation of the motor nerve to a perfused voluntary muscle led to the appearance of acetylcholine in the perfusate, the perfusion fluid containing an anticholinesterase; Brown, Dale and Feldberg (1936) that close-arterial injections of small amounts (1.5µg.) of acetylcholine produced a contraction similar to a muscle twitch. Vesicles, observed by Palade and Paley (1954), which tend to accumulate adjacent to the postsynaptic folds of the neuromuscular junction of the frog (Birks, Huxley and Katz, 1960) probably contain the transmitter and each discharges one quantum (del Castillo and Katz, 1956) into the synaptic cleft. Krnjević and Mitchell,(1961) have estimated that each nerve-ending releases about 10^{-17} moles of acetylcholine per impulse. The most direct evidence for this hypothesis is that, from homogenized guinea-pig brain, the subcellular fraction which contains similar

vesicles has a high acetylcholine content (Whittaker, Michaelson and Kirckland, 1964).

Del Castillo and Katz (1954) showed that, in frog sartorius, the end-plate current increased the conductance of the end-plate membrane for sodium and potassium, without altering its permeability to chloride, the membrane being slightly more permeable to sodium than to potassium during the action of the transmitter. An increase in the permeability of the subsynaptic membrane to cations would seem to constitute the action of the transmitter substance, acetylcholine. Katz and Miledi (1967) have indicated that transmitter release is dependent on the entry of calcium ions into the axon terminal. An excess of magnesium ions reduces the quantity of acetylcholine released per impulse (del Castillo and Engbaek, 1954) and thus causes neuromuscular block.

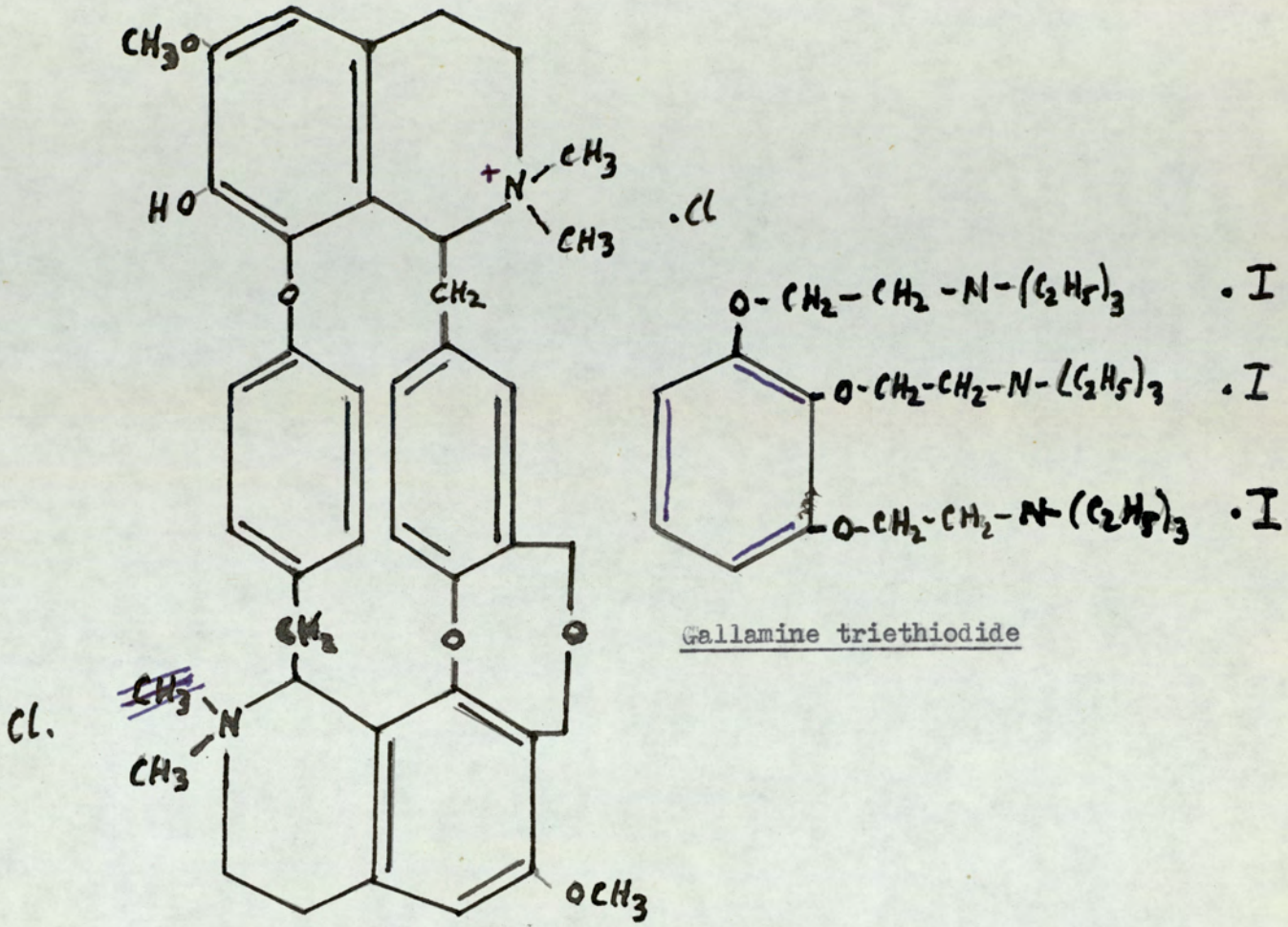
The presence of acetylcholinesterase at the neuromuscular junction was first demonstrated by Marnay and Nachmanssohn (1938). Its location in the terminal gutters on the postsynaptic membrane was established by Koelle and Friedenwald (1949) by histochemical methods. When Eccles and MacFarlane (1949) found that acetylcholinesterases increased the end-plate potential, it was clear that acetylcholine was indeed the transmitter.

Fatt and Katz (1952) suggested that acetylcholine, spontaneously released from motor nerve terminals, gave rise to miniature end-plate potentials, which were reduced in size by curare and increased by prostigmine. Katz (1962) later showed that the frequency of the miniature potentials was controlled by the condition of the presynaptic membrane, while their amplitude was governed by the properties of the postsynaptic membrane. Thesleff (1955) showed that the initial depolarization caused by acetylcholine was succeeded by neuromuscular block. Possibly, like tetraethylammonium (Stanfield, 1969), acetylcholine

inhibits the inwardly rectifying potassium channel of the muscle fibres.

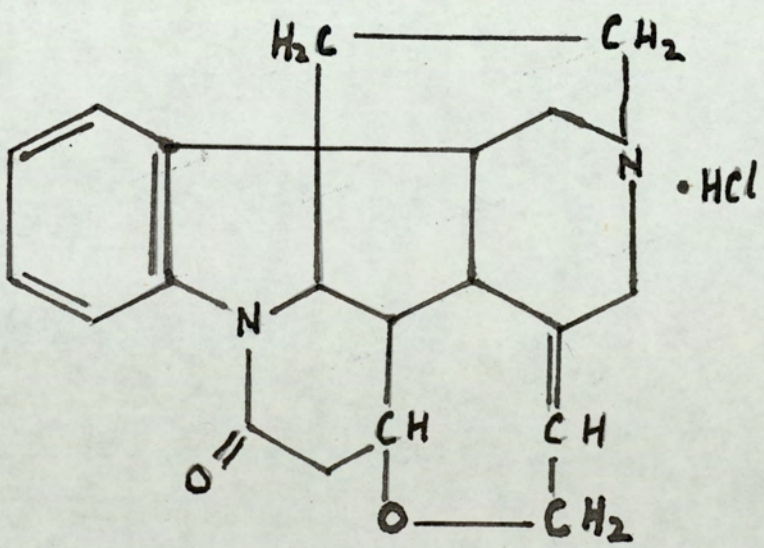
Waser (1960) represents the receptor in the muscle as a pore in the end-plate membrane, with anionic sites around the rim and cationic sites within the lumen. Bovet (1951) pointed out that, in general, competitive neuromuscular blocking agents have thick, fat and rigid molecules (e.g. d-tubocurarine, strychnine, and, to a lesser degree, gallamine) whereas depolarizing drugs have long, slender molecules (e.g. decamethonium, suxamethonium, and suxethonium). The former group (pachycurares) could seal the pore and prevent the access of acetylcholine, whereas the latter (leptocurares) might occupy the pore to simulate the action of acetylcholine. A quaternary nitrogen could depolarize the surface, while carbonyl groups attracted to cationic sites within the pore and hydrogen-bonding of alcoholic hydroxyl groups, also within the pore, possibly lead to prolonged depolarization. A bisquaternary leptocurare might bridge and deform the opening without obstructing it completely. Waser's (1960) theory is not completely satisfactory, as it would be expected that the quaternary nitrogens of d-tubocurarine, strychnine, and gallamine would depolarize, nor does it account for the distinction between a pure and a dual depolarizing blockade.

Figs. 14 and 15 illustrate the structures of competitive and depolarizing neuromuscular blocking agents, respectively, which have been used in the present work, though not all of them have been cited in this section.



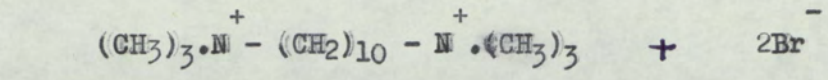
Gallamine triethiodide

d-Tubocurarine chloride

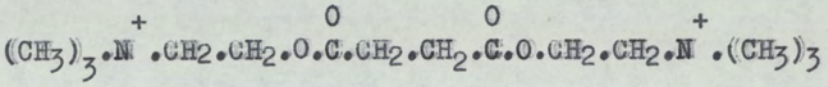


Strychnine hydrochloride

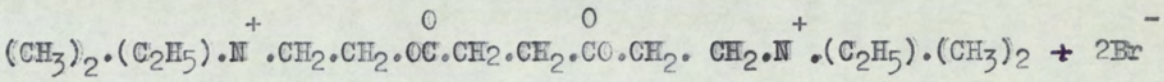
Fig. 14. Some competitive neuromuscular blocking agents.



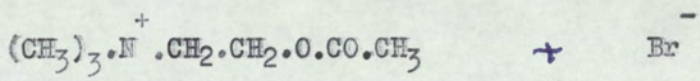
Decamethonium bromide



Suxamethonium bromide



Suxethonium Bromide



Acetylcholine bromide

Fig. 15. Some depolarizing neuromuscular blocking agents.

Veley and Walker (1909) assessed the activity of strychnine by the abolition of muscular contractions of frog sartorius. Langley (1919) showed that nicotine antagonized the paralysis of nerve cells and nerve-endings by curare and strychnine in a similar manner. That nicotine antagonized the action of strychnine on the spinal cord of the cat was confirmed by Schweitzer and Wright (1938), and the similarity of the cortical effects of curare and strychnine by Chang (1953) and Banerjee, Feldburg and Georgiev (1970). Strychnine and d-tubocurarine are also similar in that both are histamine-liberators (Crossland, 1970). The Macusi of Guyana make their curare from Strychnos toxifera (Quiggin and Wissler, 1971) and strychnine was first obtained from Strychnos ignatii (Visek, 1971), their similarity in action is thus matched by a similar distribution in plants.

Bowman (1962) prefers the term competitive for the type of block produced by strychnine, d-tubocurarine and gallamine. Rushton (1933) demonstrated that curare does not produce an observable change in excitability and strongly opposed Lapicque's (1926) theory of curarization, that the block was the result of heterochronism. This supports the finding of Grundfest (1932). Brookes and Mackay (1971) conclude that the rate of onset and offset of neuromuscular blockade by tubocurarine and gallamine are controlled by diffusion.

Small junctional potentials were detected by Burke and Ginsborg (1956a) in slow muscle fibres of the frog and they showed that the membrane resistance decreased with depolarization and increased with hyperpolarization. The average value of equilibrium potential was found to be intermediate between that corresponding to a potassium- and a sodium-selective membrane (Burke and Ginsborg, 1956b), which indicated that the small junctional potentials were the result of an increased permeability of the end-plate region to more than one ionic species. Burke (1957) demonstrated that the size of the small junctional potentials was increased by the addition of calcium and decreased by magnesium and suggested that the small junctional potentials were due to a nearly simultaneous discharge of units, which are also active spontaneously. Costantin, Podolsky and Tice (1967), using the tonus bundle of the iliofibularis of the frog, found that twitch fibres shortened many times more rapidly than slow fibres when calcium was applied, but that the spread of contraction following locally applied calcium was much greater in slow than in twitch fibres. They attributed the limitation of the spread of contraction in twitch fibres to accumulation of the metal in the rich sarcoplasmic reticulum. Evans (1971) has shown that low concentrations of saxitoxin abolish end-plate potentials in frog sartorius, but the miniature end-plate

potentials in extensor longus digitorum IV were not reduced in amplitude and that their frequency often rose dramatically after a few minutes in a high concentration of saxitoxin. By analogy, it may be presumed that the flaccid paralysis in peronaeus longus induced by depolarizing agents is associated with the abolition of the end-plate potential and that the contracture of tibialis anticus with an increase in the frequency of miniature end-plate potentials. This does not, however, throw any light on the basis of the initial potentiation of twitch fibres in either muscle or on that of any difference in sensitivity between the twitch fibres of the two.

AN ASSESSMENT OF THE PROPORTIONS OF SLOW FIBRES IN THE GASTROCNEMIUS MUSCLE OF FROGS AND TOADS

In its maximal response to a neuromuscular blocking agent of the depolarizing type, the tibialis anticus of the frog or toad undergoes a contracture, the height of which is approximately equal to its response to a single maximal indirect stimulus, whereas the contracture of the gastrocnemius is considerably less than the corresponding twitch. It was found that the height of a maximal contracture of the gastrocnemius in toads was relatively greater than that of frogs, so a comparative study was undertaken to assess the difference.

METHOD: A double myograph, employing frontal writing levers (p43c) was used in conjunction with a Keith Lucas muscle bath. An isolated nerve-muscle preparation (p. 38c), including the tibialis anticus and gastrocnemius muscles, was mounted in the bath, which contained frog Ringer (p. 46 IV) aerated by an aquarium pump. The sciatic nerve was placed over a pair of electrodes connected to a Palmer H45 square-wave stimulator, and a few drops of liquid paraffin applied to the exposed length of the nerve. Maximal stimuli (6v x 40 μsec.) were given at intervals of 20 seconds. Drugs, dissolved in frog Ringer, were added to the bath.

In order to take account both of the rate and the size of the response, planimetry was used as follows:-

(a) Assessment of a flaccid paralysis:

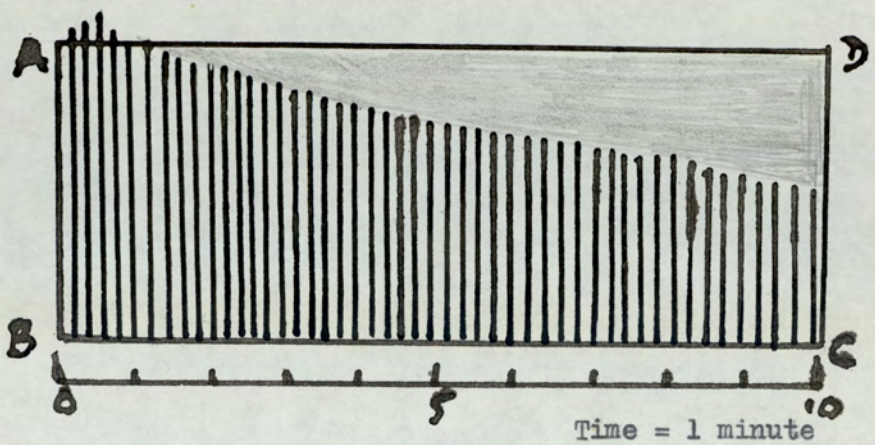


Fig. 16. Planimetry applied to a flaccid paralysis.

On the assumption that no fatigue occurs in control experiments, the twitches will maintain their height and, ignoring the initial potentiation shown in the presence of a depolarizing agent, a nil response may be indicated by the area ABCD; the extent of a flaccid paralysis by the shaded area. Expressed as a percentage, it may be said:-

$$\frac{\text{Shaded area x 100}}{\text{Area ABCD}} = \% \text{ flaccid paralysis.}$$

(b) Assessment of a spastic paralysis:

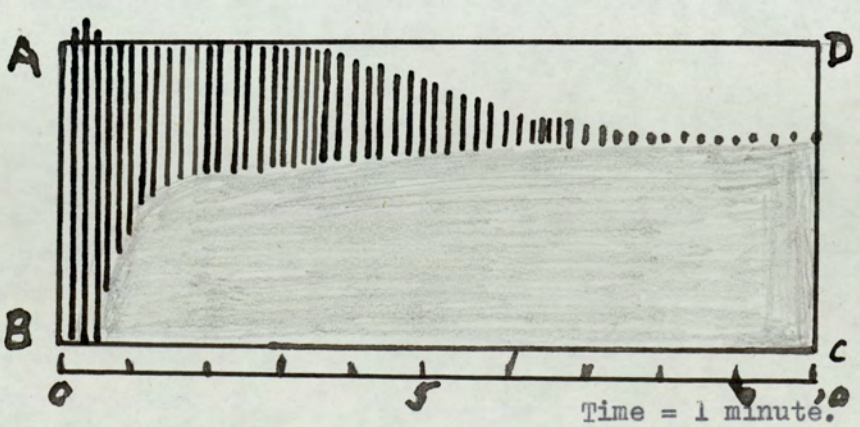


Fig. 17. Planimetry applied to a spastic paralysis.

In the same way, spastic paralysis may be expressed as a percentage achieved over 10 minutes (Fig. 17):-

$$\frac{\text{Shaded area} \times 100}{\text{Area ABCD}} = \% \text{ spastic paralysis.}$$

This value may be taken as an index of the proportion of slow fibres in a gastrocnemius muscle.

RESULTS.

(i) Frog (Rana temporaria)

		Gastrocnemius		Tibialis anticus	
		Spastic %	Flaccid %	Spastic %	Flaccid %
Control	(7)	0	4.0	0	4.2
Decamethonium 0.7 mg	(1)	22.0	14.7	62.6	4.2
Suxamethonium 0.1 mg	(4)	25.4	17.0	141.3	-
5.0 mg.	(3)	31.3	42.3	116.8	-
Suxethonium 0.3 - 1 mg.	(4)	30.1	28.6	159.6	-
1.0 mg + 1.0 mg neostigmine	(1)	48.3	40.0	165.1	-
Nicotine 1.0 mg.	(4)	15.1	60.7	22.9	52.3
5.0 mg.	(3)	20.4	57.7	44.5	48.2

Table 13. Effect of neuromuscular blocking agents of the depolarizing type on gastrocnemius and tibialis anticus of the frog (Rana temporaria). Responses measured by an Allbrit planimeter. For explanation see text.

(ii) Common Toad (Bufo bufo)

		Gastrocnemius		Tibialis anticus	
		Spastic	Flaccid	Spastic	Flaccid
		%	%	%	%
Control		0	10.0	0	8.9
Suxamethonium 1 mg.	(1)	102.0	-	107.7	-
Suxethonium 0.5 - 0.6 mg	(4)	42.0	21.5	87.0	-
1.0 - 1.5 mg	(4)	55.6	23.9	93.6	-

Table 14. Effect of neuromuscular blocking agents of the depolarizing type on gastrocnemius and tibialis anticus of the common toad (Bufo bufo). Responses measured by an Allbrit planimeter. For explanation, see text.

(iii) Green Toad (Bufo viridis)

		Gastrocnemius		Tibialis anticus	
		Spastic %	Flaccid %	Spastic %	Flaccid %
Suxamethonium 0.1 mg.	(1)	102.8	-	144.2	-
0.4 mg.	(1)	121.0	-		
Suxethonium 0.2 mg	(1)	26.9	10.1	26.1	8.7
0.4 mg	(2)	34.5	13.8	39.0	12.5
0.4 mg + 1.0 mg eserine	(2)	52.4	14.5	74.1	16.4

Table 15. Effect of neuromuscular blocking agents of the depolarizing type on gastrocnemius and tibialis anticus of the green toad (Bufo viridis). Responses measured by planimeter as explained in text.

(iv) Natterjack Toad (Bufo calamita)

		Gastrocnemius	
		Spastic	Flaccid
		%	%
Suxethonium			
0.5 mg.	(1)	26.2	14.6
1.0 - 5.0 mg.	(3)	35.9	17.9

Table 16. Effect of suxethonium on gastrocnemius of the natterjack (Bufo calamita). Responses measured by planimeter as explained in text.

Summary

		%
Frog		
Rana temporaria	(20)	25.4
Toads		
Bufo bufo	(9)	54.7
Bufo viridis	(7)	60.6
Bufo calamita	(4)	33.5

Table 17. Mean effect of neuromuscular blocking agents of the depolarizing type on the gastrocnemius muscles of frogs and toads. Data from Tables 13 - 16 collated.

DISCUSSION: The results indicate that there is a higher proportion of slow fibres in the gastrocnemius muscle of toads than in frogs which may well be related to the fact that toads walk, whereas frogs, having a higher proportion of twitch fibres, leap.

POTASSIUM-INDUCED CONTRACTURES

Sommerkamp (1928) stated that the contractures evoked by an excess of potassium salts on the iliofibularis of the frog were dependent, in largest part, on the tonus bundle, but that the non-tonic part also participated. Gasser (1930), reviewing contractures in skeletal muscles, pointed out that a frog sartorius, which is purely tetanic, could develop 25% of its maximum tetanic tension in response to excess potassium. Sandow and Kahne (1952) estimated that, in the frog sartorius, the time taken for potassium to diffuse into the deepest cells was about ten minutes, so that in his review of excitation-contraction coupling, Sandow (1965) pointed out the limitation to the use of whole muscles for a study of potassium-induced contractures. Nevertheless, it was thought worth while to demonstrate such contractures in the muscles under review here as an illustration of a means of inducing contractures in all types of muscle fibre.

METHOD: The skin was removed from one leg of a freshly-killed frog. The gastrocnemius muscle was reflected and cut away. The peronaeus longus muscle was freed from the tibialis anticus and from the tibio-fibula by dividing the connective tissue. A short piece of cotton was tied round the insertion and a longer piece of cotton round the origin of the muscle, which was then cut out and transferred to an isolated organ bath of 25 ml. capacity, filled with frog Ringer (p. 46, IV) aerated by an aquarium pump. The outer bath was filled with water at room temperature to stabilize the temperature. A standard frontal writing lever was used. The preparation of tibialis anticus was similar.

Responses of the muscle were recorded on a smoked kymograph drom. A drum-speed of 1 cm. per minute was suitable. The cycle of operations was as follows:-

At time 0	Start drum
At time 1 minute	Add (say) 2 ml. of 5% KCl
At time 2 minutes	Stop drum and wash out
Rest period	Not usually less than 10 minutes and up to 1 hour
Repeat cycle	

Acetylcholine, carbachol, suxethonium, and physostigmine were also employed.

RESULTS:

(a) Effects of neuromuscular blocking agents of the depolarizing type:

Tibialis anticus was found to respond well to acetylcholine, carbachol, and suxethonium. The action of acetylcholine was potentiated by physostigmine. Definite responses were obtained to doses of acetylcholine as low as 1 µg. in the 25-ml. bath. Using a drug-contact time of 1 minute and a rest period of two minutes, consistent responses were obtained to successive doses of 6 µg. of acetylcholine (Fig. 18). Following higher doses acetylcholine (e.g. 10 ug.) and of suxethonium (e.g. 0·3 mg.), a subsequent test dose of the depolarizing agent evoked a reduced response (Fig. 18), indicating a persistence of depolarization.

Peronaeus longus failed to respond to these agents, except for a small response to a massive dose (1 mg.) of carbachol.

Fig. 18. Effects of acetylcholine and suxethonium (Brevidil E) on tibialis anticus of frog (Rana temporaria) in 25-ml. isolated organ bath. Drug-contact time = i minute; Rest, after washing out, = 2 minutes.

(b) Effect of excess potassium on tibialis anticus:

Tibialis anticus responded well to the excess of potassium ions produced by adding 2 ml. of 5% KCl in frog Ringer to the 25-ml. bath, thus raising the concentration of the external fluid from 2·6 mM to 7.9 mM with respect to potassium. For full recovery from this, an interval of about 25 minutes was required (Fig. 19). The recovery could alternatively be followed by successive test doses of acetylcholine (Fig. 19), either procedure indicating the time needed to restore ionic equilibrium.

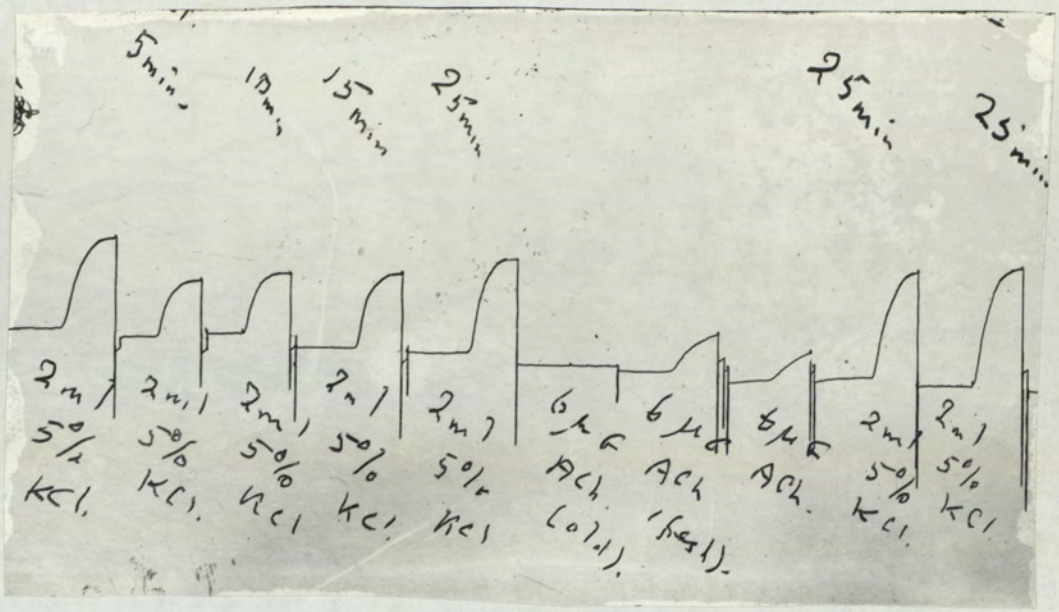


Fig. 19. Response of tibialis of frog (Rana temporaria) to potassium chloride. Drug-contact time = 1 minute; Rest, after washing out, 5 minutes, except where indicated.

(c) Effect of excess potassium on peronaeus longus:

Peronaeus longus proved less sensitive to an excess of potassium ions than tibialis anticus. It responded, however, to the addition of 5 ml. of 5% KCl in frog Ringer, thus raising the concentration in the external fluid from 2.6 mM to 16.0 mM. For full recovery from this, an interval of at least 55 minutes was required (Fig. 20). In this case, the recovery could not be followed by acetylcholine, to which the muscle is insensitive.

In these experiments, it was further noted that, whereas the tibialis anticus tended to remain in contracture, on washing out, the peronaeus longus tended to relax to a greater extent than before addition of the salt.

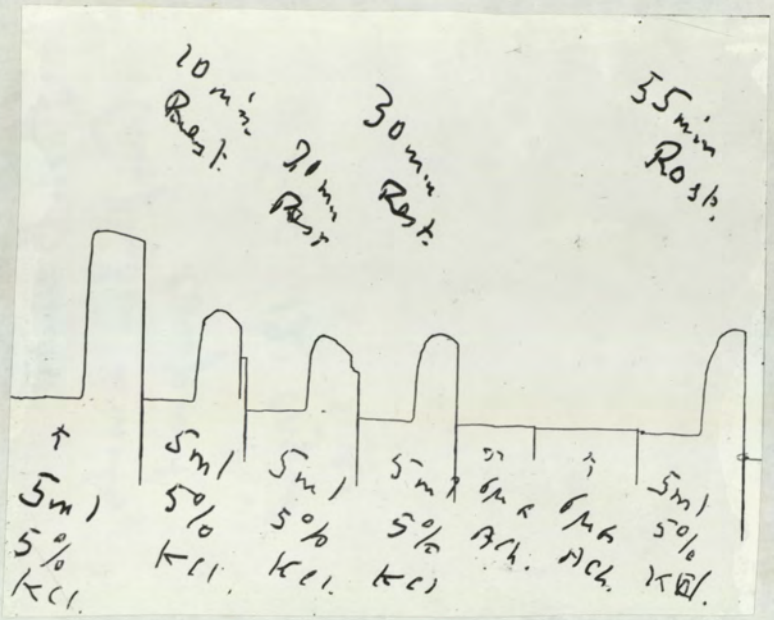


Fig. 20. Response of peronaeus longus of frog (Rana temporaria) to potassium chloride. Drug contact time = 1 minute. Rest period after washing out, as stated.

DISCUSSION: Luttgau (1963) demonstrated that a sudden increase of the potassium concentration of the external medium surrounding single twitch fibres from the semitendinosus and iliofibularis muscles of frogs from 2 mM to 190 mM caused a rapid rise in tension to a maximum level, maintained as a plateau for several seconds, followed by rapid relaxation. For single slow fibres from iliofibularis, elevation of the potassium level led to a slow increase in tension, which was maintained throughout the time of application (50 seconds). The rapid rise in tension found in tibialis anticus here is, therefore, presumably due to the twitch fibres in that muscle. A decline in tension from its peak value was seen in peronaeus longus (Fig. 20), whereas the peak tension was maintained in tibialis anticus (Fig. 19). Frank (1960b) and Luttgau (1963) pointed out the influence of calcium ions on the contractures induced by potassium. Frank (1960a) found that the maximum tension of the contracture induced by immersing a frog's

extensor longus digitorum IV in isotonic potassium chloride was about equal to the maximum tetanic tension, whereas in the sartorius the maximum tetanic tension was always greater; the former muscle corresponds in structure to tibialis anticus, the latter to peronaeus longus. Lorković (1967), using the extensor longus digitorum IV of the frog (Rana pipiens) found that the concentration of potassium needed to produce contractures increased more than threefold when the pH was lowered from pH7 to pH 5, whereas it remained unchanged on going from pH 7 to pH 9.

Hodgkin and Horowicz (1959), using fibres from semitendinosus of the frog, found that increasing the potassium in the external fluid from 2.5 to 10.0 mM produced a sudden depolarization followed by a slow drift to equilibrium value. On reducing the external potassium to its former value, it took about an hour to establish the original potential. The results obtained on the corresponding peronaeus longus muscle here are in good agreement, confirming the validity of experiments on a whole muscle, particularly one which is homogenous.

Kuffler and Vaughan Williams (1953) showed that while acetylcholine depolarized the end-plate region only, excess of potassium depolarized frog twitch skeletal muscle fibres along their whole length and assumed that, since slow muscle fibres possess numerous and diffusely distributed junctional regions that the action of acetylcholine would also be exerted along the whole length of such fibres.

T. H. Bennett
Amplitude versus
Complement
Fig 12 original
size.

PATTERNS OF CONVULSION IN THE FROG

The occurrence of cutaneous respiration enables an amphibian to maintain convulsions for a prolonged period, during which contractions of antagonistic muscles can be recorded. Characteristic patterns of convulsion are produced by individual drugs, which indicates differential action on centres co-ordinating flexion and extension. These are illustrated by strychnine, pentamethylenetetrazol (leptazol, metrazol, cardiazol), picrotoxin, insulin, bemegride and caffeine.

As mentioned earlier (p. 36), electrical methods of stimulation are open to objection. The use of convulsants reveals the responses of muscles to endogenous stimulation. Peripheral action is precluded by the ligation of the femoral artery (p. 39d), and the fact that the partial decerebration (p. 40e) did not significantly alter the pattern of convulsions was confirmed by a limited number of experiments on intact animals (p. 40f).

METHODS: The anterior part of the brain of a frog (Rana temporaria) was destroyed by pushing a seeker through the roof of the skull, in line with the posterior margin of the eyes, and turning the point forwards. The thigh was ligated behind the sciatic nerve, which immobilized the thigh and prevented injected drugs from reaching the muscles of the leg. The tibialis anticus (flexor, Fl.) and peronaeus longus (extensor, Ext.) were separated and threads attached to their tendons of insertion. A pin through the knee secured the preparation in a bath containing frog Ringer (p. 46 IV) aerated by an aquarium pump. The threads were attached, via pulleys, to Devices UF1 220-g. dynamometers and changes in tension recorded by a Devices M2 electronic recorder. Initial tensions were independently adjusted by rack and pinion mounting of

the strain gauges. The exposed length of the sciatic nerve was coated with liquid paraffin. Convulsants, dissolved in frog Ringer, were administered by subcutaneous injection into the cranial dorsal lymph sac as a single dose in 0.1 to 1.0 ml. Suitable doses were found to be : strychnine, 5mg.; leptazol, 8mg.; picrotoxin, 2mg.; globin zinc insulin, 80 I.U.; bemegride, 1mg.

A large number of preliminary experiments had been made using levers (p.41, IIb and p.43, IIc) recording on a smoked kymograph paper.

RESULTS:

(i) Strychnine: The initial effect is an increase in frequency and intensity of spontaneous movements, accompanied by the abolition of reciprocal inhibition. The onset of convulsions occurs within about three minutes of injection. The convulsions consist of simultaneous contractions of flexor and extensor, the extensor giving stronger contractions. Usually, after one or two preliminary bursts lasting a few seconds, a prolonged convulsion occurs which persists for about two minutes and is followed by shorter convulsions, ultimately leading, about 13 minutes after the injection, to single simultaneous contractions of the flexor and extensor at a frequency of about eight per minute. During the convulsions, each muscle contracts in a manner similar to that of the incomplete tetanus induced by electrical stimulation at a frequency of about ten per second. A typical example is shown in Fig. 21.

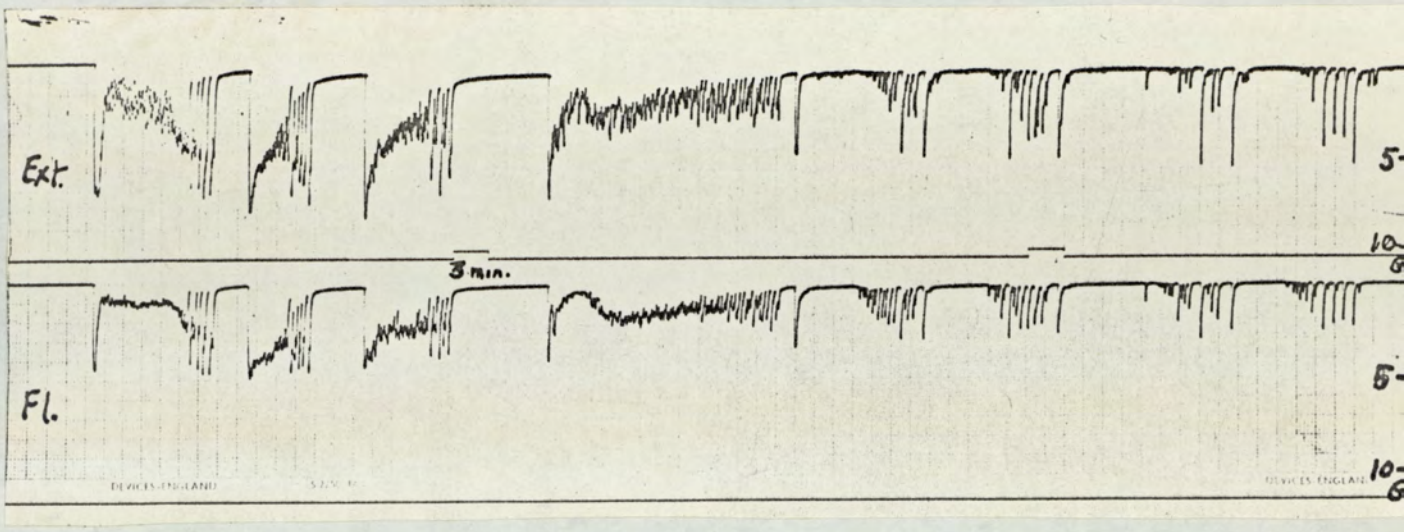


Fig. 21. Strychnine-induced convulsions in the frog (Rana temporaria). 1mg. strychnine injected into cranial dorsal lymph sac at time 0. Peronaeus longus (Ext.), above; tibialis anticus (Fl.), below. Time = 1 minute. Tension = grammes weight. Partly decerebrate preparation.

(ii) Leptazol (pentamethylenetetrazol, metrazol):

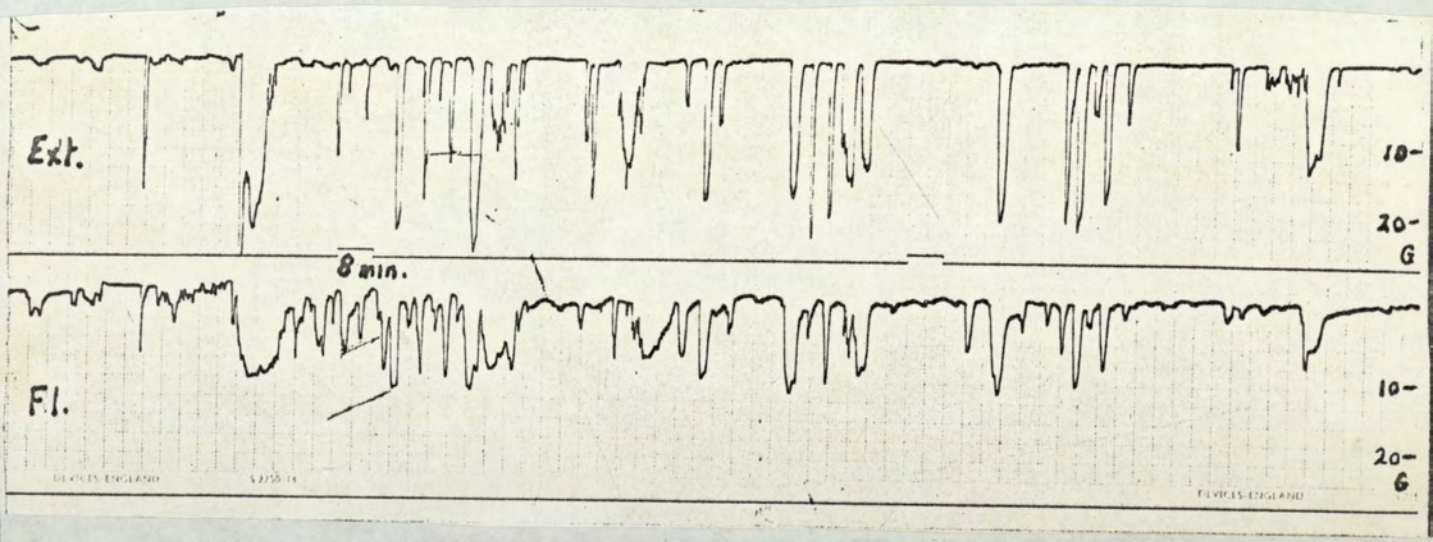


Fig. 22. Leptazol-induced convulsions in the frog (Rana temporaria). 8mg. leptazol injected into cranial dorsal lymph sac at time 0. Peronaeus longus (Ext.), above; tibialis anticus (Fl.), below. Time = 1 minute. Tension = grammes weight. Partly decerebrate preparation.

Following small doses of leptazol (1 - 5mg.), asynchronous convulsive movements of the flexor and extensor occurred. The most prolonged of these commenced about nine minutes after the injection and lasted about 22 seconds in the case of the flexor, and about 15 seconds in the extensor. The flexor was the more active, i.e. contracted more frequently, though the strongest contractions of the extensor usually developed the greater tension (Mean = 25g.) than those of the flexor (15g.). Intermittent, irregular, asynchronous convulsive movements occurred for upwards of six hours. Occasionally, such doses induced continuous convulsions; where this happened it was sometimes up to an hour after the injection and followed more typical convulsive movements.

Larger doses of leptazol (8 - 10mg.) induced convulsive movements leading to a continuous convulsion. This commenced about 8 minutes after the injection and was of the pattern illustrated in Fig. 22. The longest continuous convulsion of the flexor lasted, on average, 2 minutes, that of the extensor, about 80 seconds. The convulsion was followed by discontinuous convulsive movements, irregular in strength and frequency, occasionally leading to synchronous twitches of the strychnine pattern.

An injection of strychnine during convulsive movements induced by leptazol at first potentiated these movements but, within 3 minutes, converted them to synchronous twitches of flexor and extensor, those of the extensor being the stronger. This occurred after both small and large doses of leptazol, as here defined.

(iii) Picrotoxin. Following a dose of 0.5mg. picrotoxin, convulsive

movements of flexor and extensor developed in the first twenty minutes, then a more prolonged convulsion ensued, the mean duration of this being 113 seconds for the flexor and 39 seconds for the extensor. The movements of the muscles during the convulsive movements and convulsions were much slower than those which were induced by leptazol. A convulsion induced by picrotoxin is shown in Fig. 23. The first convulsion was followed by intermittent convulsive movements and occasional sustained convulsions with intervening periods of comparative quiescence which lasted as long as 9 minutes.

An injection of strychnine caused the replacement of the convulsive movements of picrotoxin by synchronous twitches of the two muscles in about three minutes.

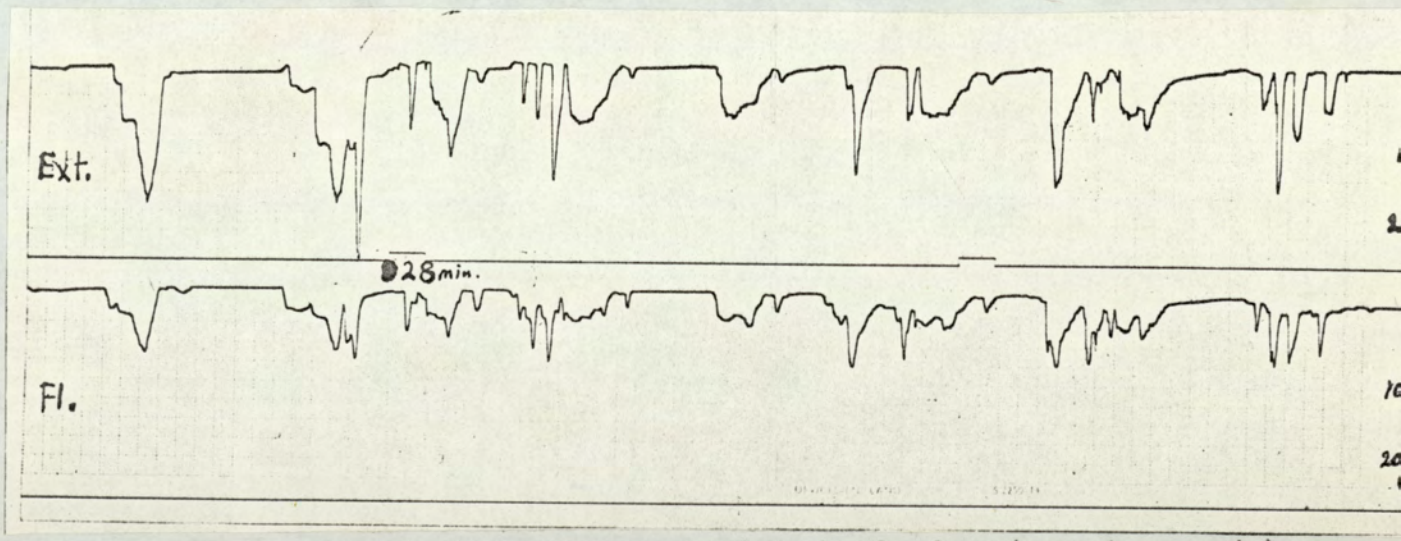


Fig. 23. Picrotoxin-induced convulsions in the frog (Rana temporaria). 0·5mg. picrotoxin injected into the cranial dorsal lymph sac at time 0. Peronaeus longus (Ext.), above; tibialis anticus (Fl.), below. Time = 1 minute. Tension = grammes weight. Partly decerebrate preparation.

(iv) Bemegride. Bemegride-induced convulsions are illustrated in Fig. 24

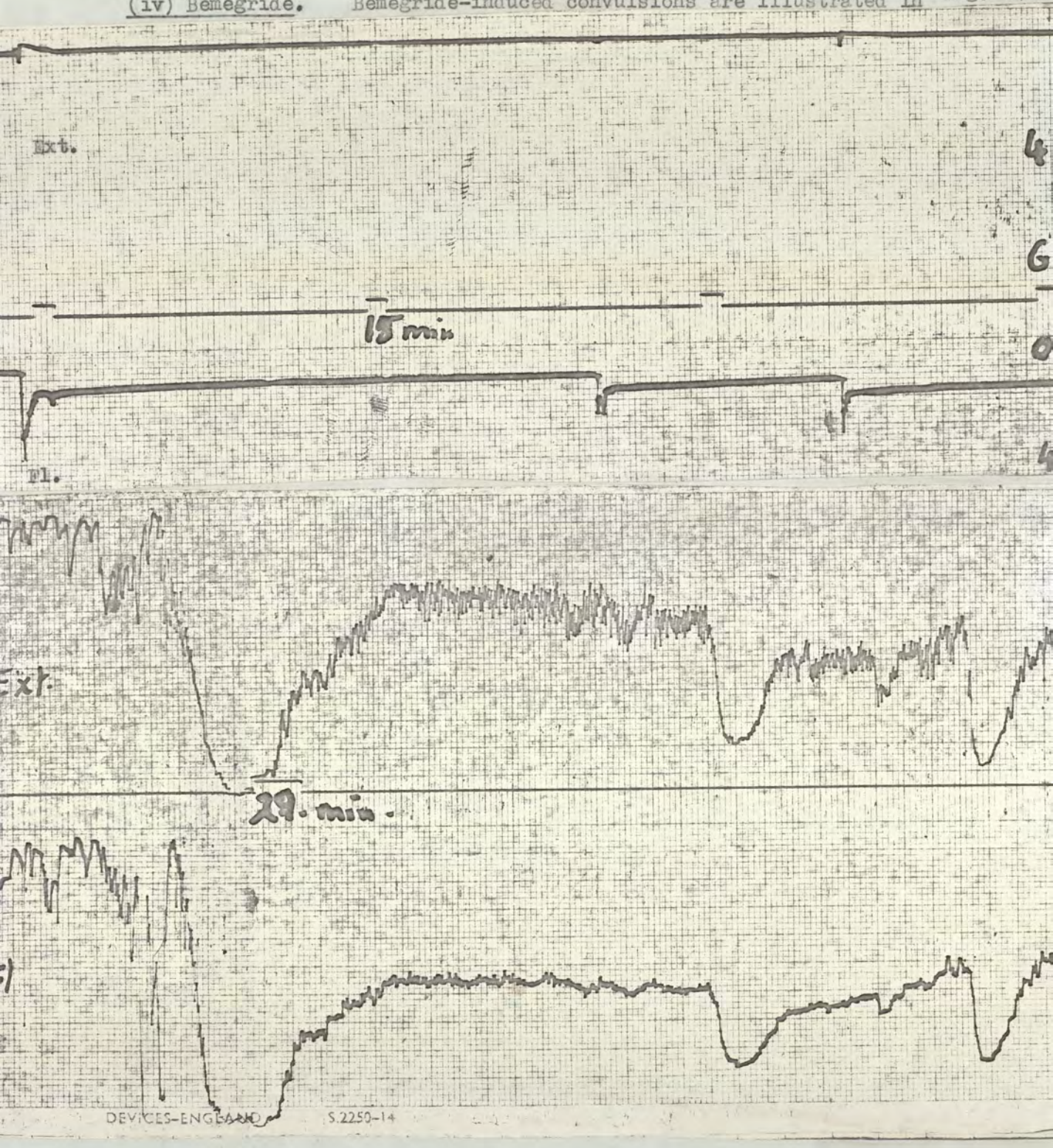


Fig. 24. Stages in the development of bemegride-induced convulsions.

1mg. bemegride injected into the cranial dorsal lymph sac at Time o.

Peronaeus longus (Ext.), above; tibialis anticus (Fl.), below.

Time = 1 minute. Tension = grammes weight. Partly decerebrate

Following the injection of 1mg. bemegride into the cranial dorsal lymph sac, spontaneous movements continued, those of the flexor being converted into convulsive movements in about 10 minutes, while those of the extensor declined to the point of complete suppression which persisted for about 20 minutes. A convulsion of the flexor commenced about 25 minutes after the injection and lasted, on average, 138 seconds, while a convulsion of the extensor commenced about a minute later than that of the flexor and persisted for a somewhat shorter period (Mean = 108 seconds). The convulsions, as was the case with picrotoxin, were followed by intermittent convulsive movements and convulsions. In this case, the periods of apparent torpor were more prolonged lasting up to 15 minutes.

(v) Insulin

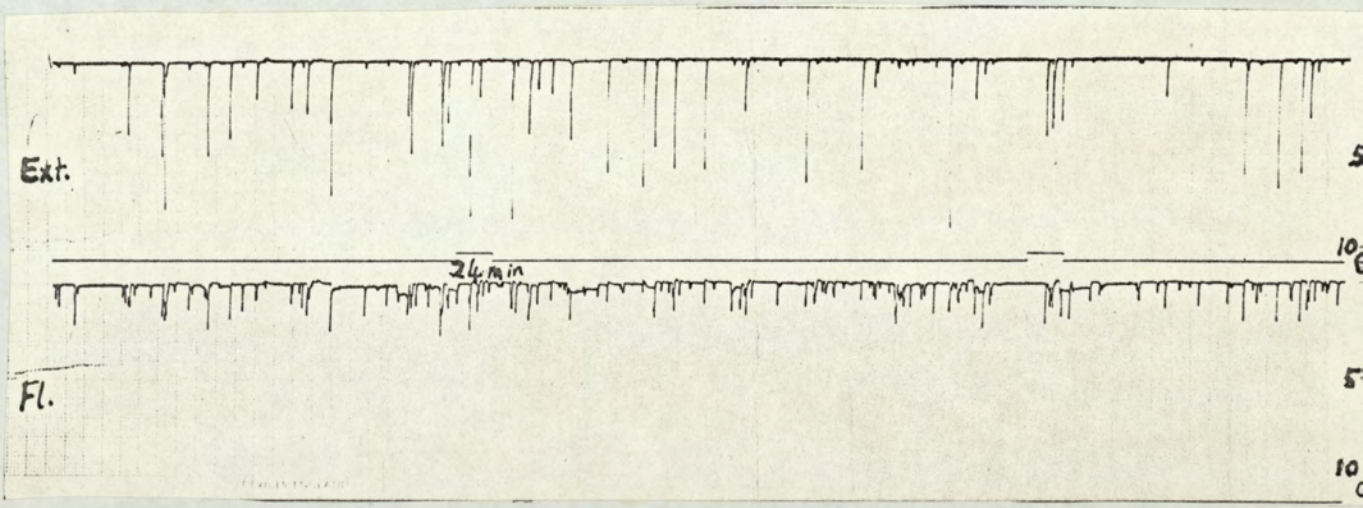


Fig. 25. Insulin-induced convulsions in the frog (Rana temporaria). 80 I.U. globin zinc insulin injected into the cranial dorsal lymph sac at Time 0. Peronaeus longus (Ext.), above; tibialis anticus (Fl.), below. Time = 1 minute. Tension = grammes weight. Partly decerebrate preparation.

A small dose (8 I.U.) of globin zinc insulin induced weak (2 - 4gm.) twitches of the flexor, which increased in frequency, achieving a rate of 5 per minute in six minutes and 18 per minute within an hour. Twitches of the extensor (up to 6 gm.) commenced six minutes after the injection but did not exceed a rate of 4 per minute in the first hour.

Folowing a larger dose (40 - 80 I.U.) of globin zinc insulin, twitches of the flexor again occurred before and were more frequent than those of the extensor. Tonic twitches, which fluctuated in strength and frequency persisted for more than three hours, the longest quiescence lasting 2 minutes. An illustration of insulin-induced convulsions is given in Fig. 25.

(vi) Caffeine. An injection of caffeine (1mg.) induced brief convulsive movements of both muscles in about ten minutes (Fig. 26), those of the flexor being the stronger. Further serial injections did not induce prolonged convulsions.

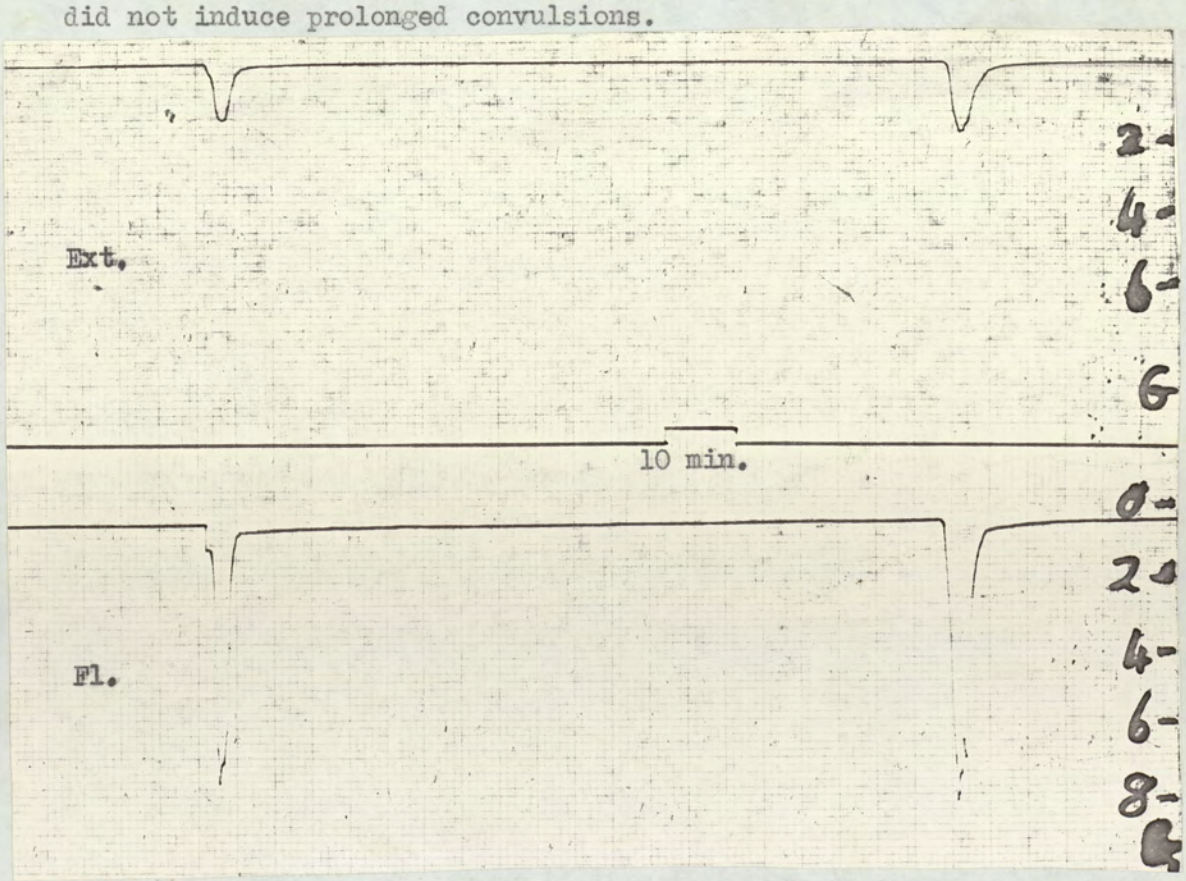


Fig. 26. Caffeine-induced convulsive movements in the frog (Rana temporaria). 1mg. caffein subcutaneously at time 0.

DISCUSSION. The actions of convulsants are frequently referred to a blocking action on presynaptic or postsynaptic inhibition. In the decerebrate preparation, two important relay centres controlling the activity of the muscles of the hindlimbs persist, the first being situated in the grey matter of the lumbar region of the spinal cord, the second in the gracile nucleus. Extensive studies have been made of the facilitation and inhibition of spinal neurones in the lumbar region of the spinal cord of the cat and of similar processes in the cuneate nucleus, which probably essentially resembles the gracile nucleus.

In synapses between neurons, transmission may be chemical, or the presynaptic cell may excite the postsynaptic cell directly by means of an electric current. The structure of chemically transmitting synapses varies in detail, but two particular features are common to them all: (a) synaptic vesicles are present in the presynaptic nerve-endings, and (b) a cleft, about 200A° across, separates the pre- and post-synaptic cells. The pre- and post-synaptic membranes are usually thickened in places, and these thickenings are probably of special importance in the process of transmission.

Synaptic excitation in mammalian spinal motoneurons: In mammals, the cell bodies of the motoneurons innervating the muscles of the limbs lie in the ventral horn of the spinal cord. In the foetus, the cells are randomly dispersed, but in the adult they are grouped according to function, e.g., flexion, extension, abduction, adduction. The axons pass out to peripheral nerves via the ventral roots. The cyton, or cell body, is about 70μm. across and bears a number of finely branching dendrites. The surface of the cyton and of the dendrites is covered with small presynaptic nerve-endings (terminal

boutons), which present the typical features of chemically-transmitting synapses. Many of these terminal boutons are the endings of Group Ia afferent fibres from muscle spindles. Stretching the muscle activates these Group Ia fibres, which may then excite the motoneuron and cause a contraction of the muscle. This constitutes a monosynaptic reflex (Fig. 27).

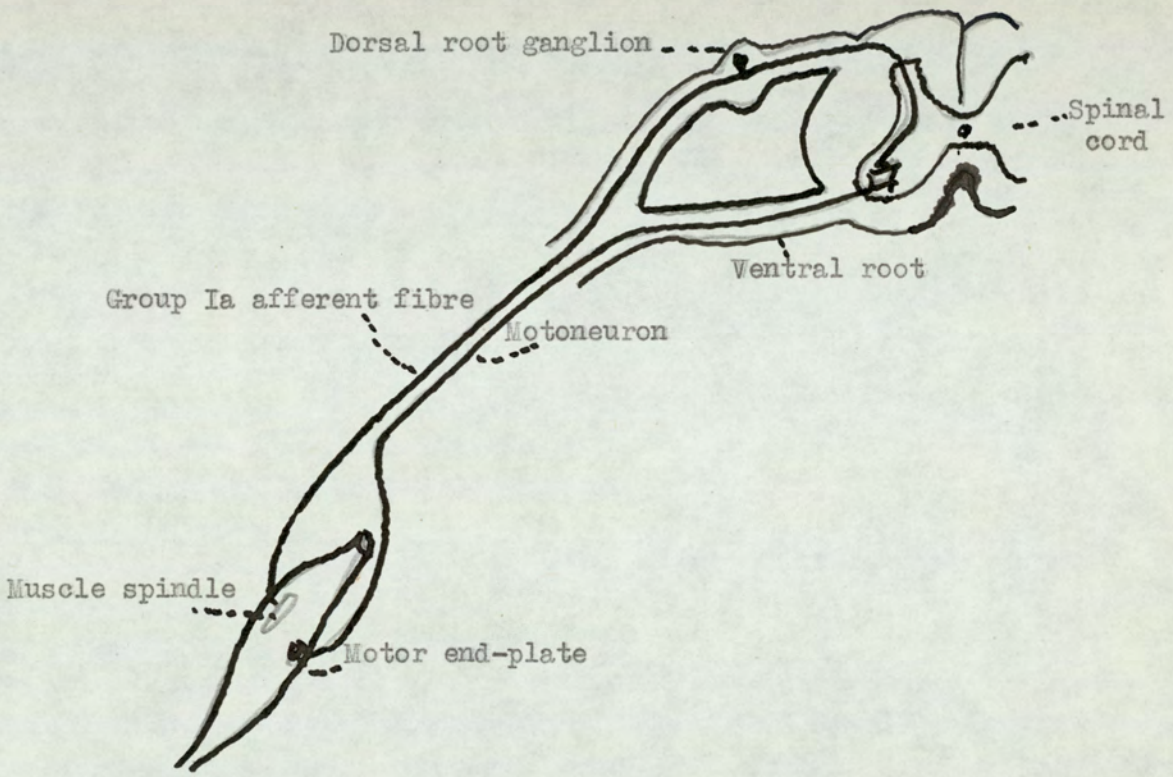


Fig. 27. Diagrammatic representation of the monosynaptic stretch reflex system.

The diagram is grossly simplified, there being many stretch receptors and afferent and efferent neurones associated with each muscle.

Most of our knowledge is based on studies of the synaptic responses of motoneurones recorded from the lumbar region of the spinal cord of the cat. Brock, Coombs and Eccles (1952) first developed the technique of using intracellular electrodes for examining motoneurons. Maps showing the location of various motoneuron groups associated with

particular muscles have been prepared, e.g. Romanes (1951), which enable the microelectrode to be positioned approximately. The identity of a motoneuron is accurately established by stimulating the motor nerve fibres of a particular muscle and observing the monosynaptic response in the neuron. The ventral roots may be cut to prevent antidromic stimulation of the motoneuron.

Motoneurons have a resting potential of about 70mV. Depolarization of the membrane by about 10mV. results in an action potential, which is propagated along the axon. Experiments involving the injection of various ions into the motoneuron, via microelectrodes, indicate that, as in the giant squid axons, the resting potential is slightly less than the potassium equilibrium potential. The action potential is caused by an increase in sodium permeability, and the ionic concentration gradients necessary for these potentials are produced by a Donnan equilibrium system in conjunction with the active extrusion of sodium ions.

Excitatory postsynaptic potentials: Excitatory postsynaptic potentials (EPSPs), produced by stimulating the nerve supplying a muscle by single shocks after section of the motor roots, may be recorded by means of a microelectrode inserted into an appropriate motoneuron (Coombs, Eccles and Fatt, 1955a). The form of the response is similar to that of an end-plate potential (EPP) in a curarized frog muscle fibre: a fairly rapid rising phase, followed by a slower decay. The size of this response is however, in this case, proportional to the intensity of stimulation, and therefore to the number of presynaptic fibres which are active. This property is known as spatial summation. If the EPSP is large enough, a propagated action potential is set up. Two EPSPs, neither of which is alone capable of setting up a propagated

action potential, are produced within a sufficiently short time of each other, they may produce an action potential. This phenomenon is called temporal summation.

The form of the EPSP suggests that, like the EPP of muscle, it is produced by a brief period of ionic flow induced by the transmitter substance. Most of the current flow occurs in the first millisecond, but there is a prolonged tail, indicating continuation of transmitter action at a low level during the falling phase of the EPSP.

If the cell membrane is progressively depolarized, the EPSP decreases in size and eventually becomes reversed in sign. Thus small depolarizations render the cell more excitable than in the normal resting condition, but larger depolarizations make the cell electrically inexcitable.

The ionic mechanism of the EPSP in motoneurons has not been as fully investigated as that of the EPP. It is technically difficult to alter the ionic environment of motoneurons. The transmitter released from the Group Ia sensory fibres involved in the monosynaptic reflex has not been identified.

Inhibition in mammalian spinal motoneurons: To obtain co-ordinated movements, when a muscle contracts its antagonist relaxes. In the monosynaptic stretch reflex, this is brought about by inhibition of the flexors through a system known as the direct inhibitory pathway. This is illustrated in Fig. 28.

Group Ia afferent fibres from stretch receptors in the extensor muscle synapse in the ventral horn with motoneurones innervating that muscle and, to a lesser degree, with its synergists, that is other muscles producing extension at the same joint. These afferents also synapse with small interneurones which form connections with the

motoneurones of the flexor. It is the interneurons which exert the inhibitory action on the flexor. The inhibitory action can be studied by inserting a microelectrode into a motoneuron and stimulating the Group Ia afferents from an antagonistic muscle. The response consists of small hyperpolarizing potentials, known as inhibitory postsynaptic potentials (IPSPs).

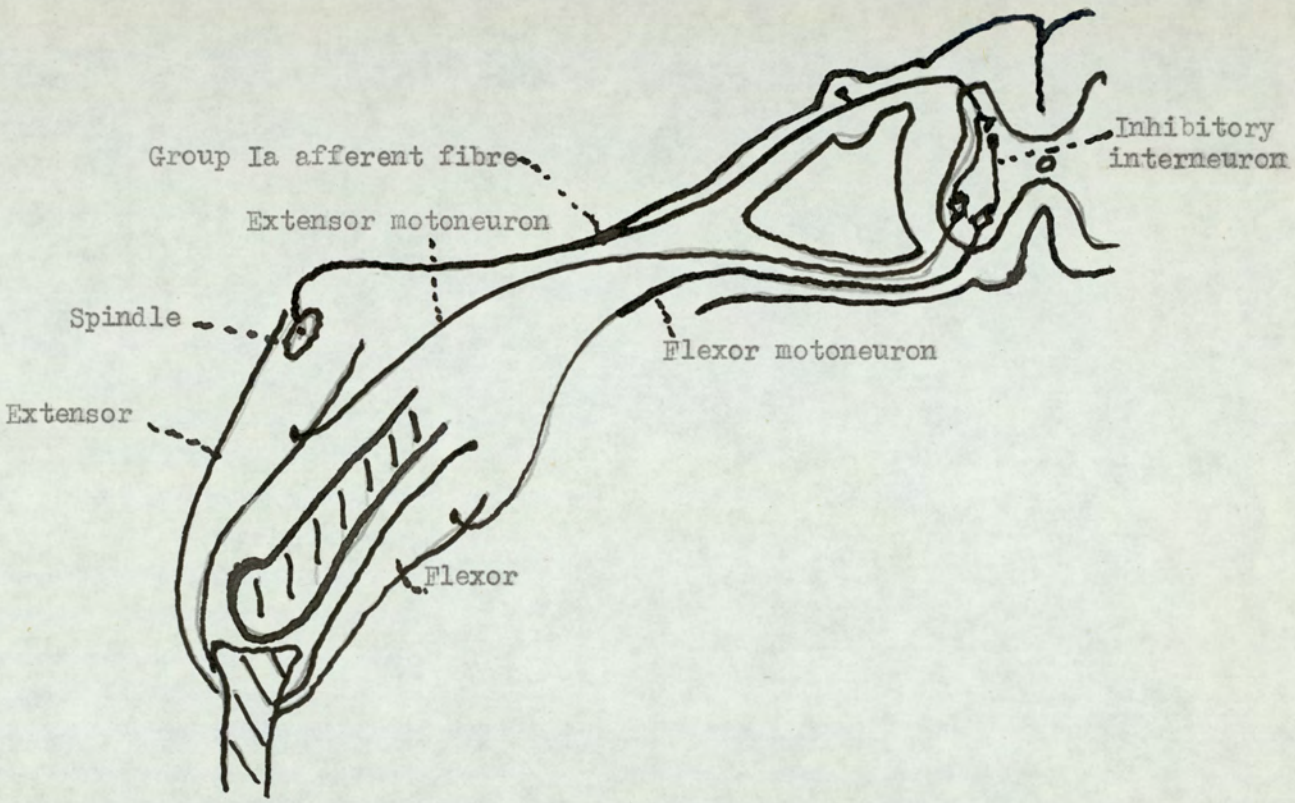


Fig. 28. Diagrammatic representation of the direct inhibitory pathway.

Apart from being hyperpolarizing rather than depolarizing, the shape of the IPSP is similar to those of the EPSP and the EPP. That it is caused by a brief change in the ionic conductance of the membrane which causes the initial depolarization, followed by a passive decay of the charge on the membrane capacitance, has been verified by analysis of the curve (Coombs, Curtis and Eccles, 1956) and, more directly, by means of voltage-clamp technique (Araki and Terzuolo, 1962). Coombs, Eccles and Fatt (1955b) have demonstrated that IPSP increases on

depolarization, but decreases and then reverses in sign on hyper-polarization.

By altering the ionic concentrations inside a motoneuron, Coombs, Eccles and Fatt (1955b) found the results most clear-cut after chloride injections, and it may be deduced that the inhibitory transmitter substance causes an increase in the permeability of the postsynaptic membrane to chloride ions. It is probable, though the evidence here is less conclusive, that the IPSP also depends on an increase of potassium conductance in the membrane.

Presynaptic inhibition: A mechanism in the central nervous system by which an interneuron reduced the polarization of a presynaptic terminal could reduce the size of the response in the postsynaptic cell, since the amount of transmitter released is related to the degree of depolarization of the motor terminal. A depolarization of the nerve terminal by a few millivolts will considerably reduce the amount of transmitter released. Evidence that presynaptic inhibition exists has been adduced by Eccles, Eccles and Magni (1961) and Eccles, Magni and Willis (1962) showed that dorsal root afferent fibres subjected to presynaptic inhibition are depolarized, and that the depolarization follows a time-course which coincides with that of the inhibitory action, i.e. it begins about 2·5msec. after the inhibitory volley enters the cord, reaches a maximum about 15msec. later, and lasts for 200msec. or over.

A central delay of 2·5msec. suggests a polysynaptic pathway. The long duration of presynaptic inhibition could result from multiple firing of the interneurons involved (reverberating interneurones).

A simple representation of the mechanism of presynaptic inhibition is given in Fig. 29.

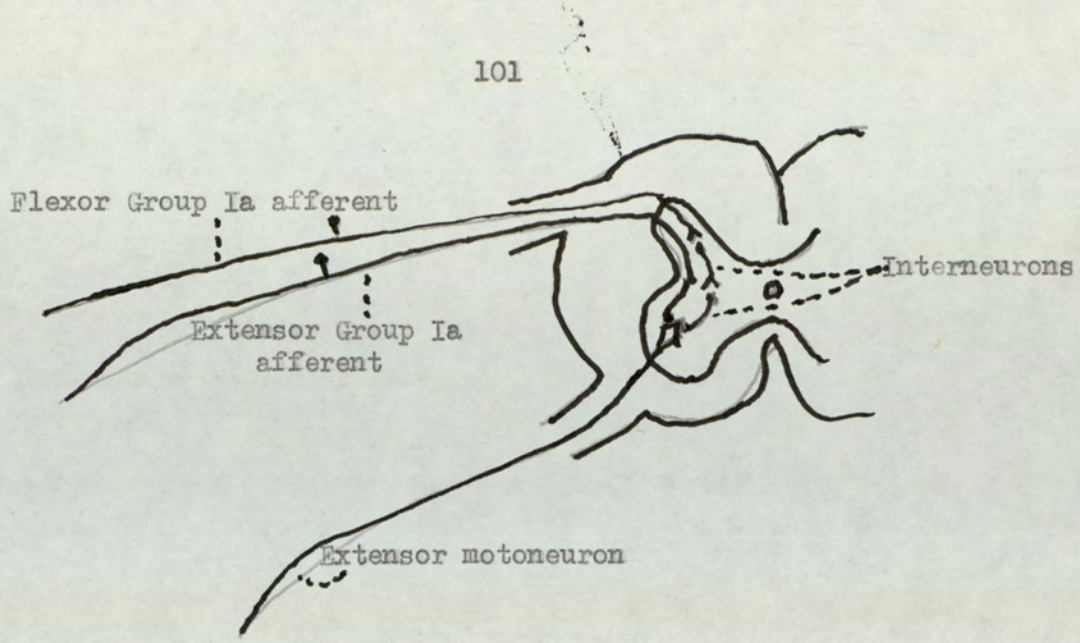


Fig. 29. Diagrammatic representation of presynaptic inhibition.

Electrically transmitting synapses: An excitatory electrically transmitting synapse is one in which the postsynaptic cell is directly excited by electrotonic currents accompanying an action potential in a presynaptic cell. There are three features to ensure an adequate current-density in the postsynaptic cell: (a) the synaptic cleft is narrow; (b) the resistances of the pre- and post-synaptic membranes are low, and (c) the size of the postsynaptic cell is not much greater than that of the presynaptic cell. The advantage of electrical transmission is the reduction of the synaptic delay, which may prove negligible. Such synapses have not been identified in amphibians or mammals but could be served by similar facilitatory and inhibitory mechanisms, indeed an inhibitory mechanism involving interneurons has been described in the goldfish (Furukawa and Furshpan, 1963).

The gracile and cuneate nuclei: In addition to those which end in the monosynaptic and short interneuronal pathways, a large number of afferent fibres which enter the spinal cord by the dorsal roots of the lumbosacral region pass to the gracile nucleus, sending collateral branches to the grey matter as they ascend. Being constantly

joined by ascending fibres from higher segments, the fasciculus gracilis becomes medially displaced and marked off from the cuneate fasciculus by an incomplete dorsolateral septum. The fibres of the gracile and cuneate fasciculi both subserve the senses of touch, pressure, and kinaesthesia so, though the cuneate nucleus has been the more frequently investigated, it is probable that no essential difference exists between the two centres. Relay neurons from the dorsal column nuclei decussate by passing ventromedially in the medulla oblongata as the internal arcuate fibres and then pass rostrally as the medial leminiscus to terminate in the lateral part of the ventroposterior nucleus of the thalamus, whence a third and final neuron relays impulses to the postcentral gyrus of the cerebral cortex. This final neuron is destroyed in the partly decerebrate preparation.

Cutaneous afferent volleys were found by Therman (1941) to produce on the surface of the cuneate nucleus an initial negative (N) wave followed by a large positive (P) wave, which had a time-course resembling the depression of synaptic transmission. Magni et al. (1959) recorded similar N and P waves after electrical stimulation of the contralateral sensorimotor cortex. The synaptic mechanism producing these surface potentials in the cuneate nucleus were investigated by Andersen et al (1964 a,b,c,d) who showed that the N wave was the result of synaptic depolarization of relay cells between the cuneate nucleus and the thalamus, whereas the P wave was due to prolonged depolarization of the presynaptic terminals of the cuneate tract fibres. The general features of presynaptic and postsynaptic inhibition in the cuneate nucleus were comparable to those observed in neurons of the spinal cord. Using the technique of antidromic invasion from the medial leminiscus, it was found (Andersen et al, 1964b) that some

neurones, regarded as relay cells, responded by a spike potential, whereas others, regarded as interneurones, failed to respond.

Towe and Jabbur (1961) found that a considerable number of the neurons in the cuneate nucleus could be either excited or inhibited by stimulation of the pericrucial cortex. The cortically excited neurones occupied deep and rostral sites in the dorsal column nuclei, the excitation being transmitted entirely by the pyramidal tracts. The cortically inhibited neurones were placed more superficially and inhibition involved an additional route.

Galindo, Krnjević and Schwartz (1967) established that γ-aminobutyric acid (GABA) acted as a potent inhibitor of neuronal excitation in the cuneate nucleus of cats. By stimulating through the central barrel of a five-barrel micropipette and recording of the antidromic action potentials in the superficial radial nerve, Galindo (1968) showed that direct applications of GABA and glycine depressed the terminals, an action which was reduced by picrotoxin and abolished by strychnine. The same author (Galindo, 1969) demonstrated that iontophoretic release of GABA and glycine suppressed postsynaptic excitation and synaptic transmission through the cuneate nucleus. The effects of GABA were specifically blocked by iontophoretic picrotoxin while strychnine specifically blocked the effects of glycine. These experiments suggested that the type of synaptic inhibition susceptible to the administration of picrotoxin may be mediated by GABA. Aprison and Werman (1965) have established that dorsal root fibres and columns contain substantial amounts of glutamate. Evidence from Davidson and Southwich (1971) indicates that GABA has a dual action in the cuneate nucleus, not only depressing the excitability of postsynaptic neurons, but also increasing the excitability of primary afferent terminals in a manner which might be expected of a presynaptic inhibitory transmitter.

The distribution and function of chemically-transmitting synapses: The development of specific and sensitive histochemical fluorescent methods for the cellular localization of dopamine, noradrenaline and 5-hydroxytryptamine enabled neurons which store these substances to be demonstrated and mapped, and facilitated the study of the formation, release, and inactivation of the transmitters (Hillarp, Fuxe and Dahlström, 1966).

Dopamine nerve cells are present, almost exclusively in the mesencephalon and are thought to terminate mainly with dendrites of the relaying neuron. They are characterized by very fine varicose terminals, the varicosities probably making the synaptic contacts and being structures for the synthesis, storage, and release of the amine.

Most of the noradrenaline neurons are probably situated in the pons and medulla oblongata and may terminate in contact with either dendrites or cell bodies. Some of the noradrenaline neurones extend as far as the lumbosacral region of the spinal cord, giving off collaterals as they descend, and travelling in the ventral and lateral funiculi; others have ascending fibres, which traverse the dorsal part of the reticular formation to terminate in the hypothalamus, thalamus, and cortex. By the iontophoretic application of noradrenaline to brainstem neurons in the decerebrate cat, Boakes et al (1971) described four types of effect: short-lasting inhibition; long-lasting inhibition; excitation, and a biphasic response consisting of short-lasting inhibition followed by excitation. Loizou (1971) showed, by inhibiting tyrosine hydroxylase, that dopamine-containing neurons were depleted faster and more completely than noradrenaline-containing neurons. Electrical stimulation of the pons or mesencephalon accelerated the depletion in both types.

The 5-hydroxytryptamine neurones are located mainly in the

mesencephalon and send fibres to the hypothalamus, thalamus and neocortex; those which descend to the spinal cord derive from cell bodies in the medulla oblongata. A detailed description of the distribution of the above neurons has been given by Fuxe (1965).

Acetylcholine probably acts as the transmitter at many synapses in the central nervous system. It has been established as such at the junctions between motor axon collaterals and Renshaw cells in the spinal cord. Eccles, Eccles and Fatt (1956) showed spontaneous activity of these cells following close-arterial injections of acetylcholine and nicotine. Later, Curtis and Eccles (1958) obtained responses to these drugs by iontophoretic application in the vicinity of the cells. Kuno and Rudomin (1966) have demonstrated that antidromic stimulation of motor nerves in the cat causes a release of acetylcholine in the spinal cord. Curtis and Ryall (1964) found evidence for muscarinic, as well as nicotinic, receptors on Renshaw cells, and Krnjević and Phyllis (1963) have been able to demonstrate, by iontophoretic application, that there are neurons in the cerebral cortex sensitive to acetylcholine which have muscarinic receptors. In the brain stem, Bradley and Wolstencroft (1965) found that muscarinic receptors were associated with inhibition, whereas nicotinic receptors produced excitation.

There is evidence that gamma-aminobutyric acid acts, not only in the cuneate nucleus, but also in the cerebellum and cerebral cortex (Curtis and Watkins, 1965; Hebb, 1970; Krnjević, 1970).

Glycine may also act as an inhibitory transmitter, particularly in the mammalian spinal cord (Curtis, Hosli and Johnston, 1968; Hebb, 1970).

The action of a neurotransmitter may be terminated (a) by simple diffusion from the synaptic cleft and dilution in the extracellular fluid, as possibly in molluscan ganglia operated by 5-hydroxytryptamine

(Gerschenfeld and Stefani, 1968); (b) by enzymatic conversion to inactive metabolites, familiar in the case of acetylcholine, which is hydrolysed by acetylcholinesterase, or (c) by removal of the transmitter substance by a membrane transport system close to the synaptic cleft, such a system being called an uptake mechanism. The uptake mechanisms, particularly in respect to noradrenaline, were described by Iversen (1971) in the third Gaddum Memorial Lecture. It is suggested that the transmitters are taken up and transferred to storage vesicles [redacted] within the presynaptic cells, without significant metabolic destruction or wash-out, and that this is the principal mechanism for their inactivation.

The kinetics of the uptake mechanisms have been studied in homogenates of areas of the central nervous system rich in a particular transmitter. Noradrenaline-neurons take up the naturally-occurring stereoisomer preferentially, whereas dopamine-neurones will accumulate noradrenaline or dopamine, having a higher affinity for dopamine but being nonselective as between the stereoisomers of noradrenaline (Colburn, et al., 1968; Snyder and Coyle, 1968). Iversen (1971) suggests that noradrenaline may be taken up into the synaptosomes of the nerve terminal for storage and re-use or, to a lesser extent, into the postsynaptic cell in which it will be metabolically degraded.

The action of convulsants: It is clear that the diversity of pattern exhibited in the convulsions induced by individual convulsants cannot be adequately explained merely by stating that a particular agent removes either presynaptic or postsynaptic inhibition. The above survey of the mechanisms for facilitation and inhibition of neurons and for the uptake, storage, and release of a variety of transmitter substances affords a wider basis for study.

It is likely that the neurones which constitute the grey matter of the brain in the various ganglia and nuclei, like those in the ventral horn of the spinal cord, form functional groups and that each group is responsible for the co-ordination of activity such as flexion and extension. Where convulsants, as is invariably the case with those studied here, show a differential activity on the centres controlling a flexor and extensor muscle, this co-ordination is likely to be affected though not necessarily abolished. The grouping of the neurons in all parts of the central nervous system is probably a feature of development and studies such as that on the maturation of convulsogenic activity induced by leptazol in the albino rat by de Casrilevitz, Englehardt and Esberard (1971) confirm this view.

Strychnine: Sherrington (1906) stated that, under strychnine, practically all the skeletal muscles might be reflexly thrown into simultaneous contractions and that this included antagonistic muscles. Dusser de Barenne (1933) maintained that, in the frog, the legs were in extension during strychnine poisoning because of the mechanical predominance of the extensors over the flexors in these limbs. While the peronaeus longus is the stronger muscle, it was observed here that an administration of strychnine during convulsions induced by leptazol, picrotoxin, or bemegride resulted in a change to synchronous twitches, in which the tension developed by the extensor was markedly increased. This suggests a preferential action of strychnine on centres controlling extension. Whitney (1958) precludes tetanic activation of the individual fibres of a muscle as a feature of normal muscular activity. Bishop and Clare (1957) however reported that, shortly before the stage of intoxication by strychnine at which spontaneous spikes appear in sensory fibres, a second response may

follow some of the stimuli, which suggests repetitive firing to one stimulus. In the early phase of strychnine convulsions, it may well be that centrifugal stimulation occurs at frequencies sufficient to produce a tetanus. The similarity of the early response to that induced by repetitive stimulation at a rate of about 10 per second has been mentioned above (p. 88).

In the later stages of strychnine poisoning, the convulsions become discontinuous, ultimately giving way to synchronous twitches and evidence has been adduced that this break up may be due to a defensive release of catecholamines (Bennett, 1970, 1972). Lloyd (1941) demonstrated that impulses arriving at motoneurons directly are ineffective unless the dorsal interneuron pools are active, hence repetitive volleys descending the cord during strychnine poisoning could become blocked by inactivation of the interneurons and convulsions made discontinuous.

Curtis (1962) found the membrane potentials and EPSPs of motoneurones were not affected by concentrations of strychnine sufficient to abolish IPSPs. Eccles, Schmidt and Willis (1963) stated that, in contrast to its specific depressant action on postsynaptic inhibition, strychnine usually increased presynaptic inhibition, an effect attributed to enhanced transmission along the polysynaptic pathways responsible for presynaptic inhibition. Llinas (1964) reported that, when direct inhibition was almost completely prevented by strychnine, reticular inhibition of the monosynaptic pathway of quadriceps neurons was practically unaltered, although the effectiveness of postsynaptic inhibitory action (hyperpolarization) was reduced. Lux and Pollen (1966) found that the initial action potentials in strychminized cells of the motor cortex of the cat could be as brief as those recorded from normal cells and were often followed by the primary hyperpolarizing action potential, and reported the absence of obvious effects of

this drug on passive membrane properties. Shende and King (1967) found that strychnine had no significant effect on presynaptic afferent depolarization in the trigemminal brain stem nuclei of the squirrel monkey.

Strychnine is reported as antagonizing glycine mediated inhibition in the spinal cord (Curtis, Duggan and Johnston, 1971). To study the effect of the absence of such inhibition on the spinal acetylcholine content, Nistri and Pepeu (1972) demonstrated that, in the spinal cord of winter frogs, strychnine caused a reduction in the acetylcholine content within ten minutes, but that the level returned to normal in about half an hour and was significantly elevated four hours after the subcutaneous injection, while convulsions continued. Strychnine has been found to produce only a modest increase in the output of acetylcholine into the perfused cerebral ventricles (Beleslin, Polak and Sproull, 1965). Elliott and Hobbiger (1959) reported that there was no demonstrable antagonism by gamma aminobutyric acid against lethal doses of strychnine in mice, and Harris, Hopkin and Neale (1973) that strychnine had no significant effect on the uptake of GABA by cortical slices. Presumptive evidence was adduced (Bennett, 1970) that strychnine convulsions were not mediated by pathways in which serotonin is the transmitter.

Leptazol (Pentylenetetrazol, Metrazol, Caridiazol): Leptazol acts on the whole of the central nervous system. By close-arterial injections into various cerebral vessels, Jolly and Steinhouse (1956) demonstrated that the higher centres were much more sensitive to the drug than centres in the pons and medulla oblongata. The sensitivity of the spinal cord is low (Hahn, 1960). de Casrilvitz, Engelhardt and Esberard (1971) observed that, in the rat, tonic seizures were seen at

birth; myoclonic jerks developed by the second week, and full myoclonic seizures and catalepsy of the adult pattern were established at three weeks of age. They suggested that the neuromechanisms responsible for tonic seizures and clonic jerks were located in the brain stem and spinal cord, while those responsible for myoclonic seizures and catalepsy were situated at the striato-thalamo-cortical level.

Purpura and Grundfest (1957) suggested that leptazol acted chiefly by stimulating excitatory synapses, but Boyd, Merritt and Gardner (1966) have shown that it blocks presynaptic inhibition of the cuneo-thalamic relay cells. Killam (1962) considered that there was insufficient evidence to substantiate a direct action of leptazol on the reticular formation of the brain stem. Lewin and Esplin (1961), investigating the spinal excitatory action of leptazol, found that monosynaptic activity in the peroneal nerve was reduced, while polysynaptic activity in the nerve to gastrocnemius was enhanced, and suggested that the drug increased the intensity and duration of evoked Renshaw cell discharges. Feldberg, Hall and Reit (1966) found that leptazol, perfused through the cerebral ventricles of the cat, caused shivering, which they attributed to an action on the hypothalamus. By measuring cuneate nucleus surface potentials and antidromic spike potentials, Banna and Hazbun (1961) deduced that leptazol blocked afferent terminal depolarization and reduced presynaptic inhibition. They found that it sometimes decreased the size of the postsynaptic discharge in the medial leminiscus and exerted a less pronounced and more erratic effect than picrotoxin on presynaptic inhibition. Fukuda, Walanabe and Ito (1969) reported that leptazol induced an electromyographic response in the gastrocnemius with a longer latency following stimulation of the ipsilateral peroneal nerve in intact young chickens. After microinjections of leptazol

into the cerebral cortex of cats, Bannerjee, Feldberg and Georgiev (1970) noted that spike discharges continued for only a few minutes, whereas with strychnine they persisted for an hour, and with picrotoxin for several hours. Spike discharges were obtained from several areas of cortex, but not from the caudate nucleus, thalamus, or hypothalamus.

It has been suggested (Bennett, 1970) that leptazol acts primarily through pathways in which serotonin is the transmitter, since convulsive movements similar to those induced by small doses of leptazol were obtained as a consequence of serial injections of 5-hydroxytryptophan, administered subcutaneously to partly decerebrate frogs. Boyd, Merritt and Gardner (1966) have shown that leptazol did not reduce the uptake of gamma-aminobutyric acid by rat cerebral cortex significantly. Mitchell (1963) reported that leptazol increased the release of acetylcholine from the cerebral cortex of the sheep and this was confirmed, in the cat, by Beleslin, Polak and Sproull (1965). Hemsworth and Neal (1968) found that leptazol increased the release of acetylcholine from rat cerebral cortex slightly more than strychnine but much less than picrotoxin. Somewhat at variance with these findings, Sharkawi (1972) found that leptazol did not affect the ability of slices of cerebral cortex from rats to form acetylcholine.

Picrotoxin: Schriever and Perschmann (1935) deduced that the midbrain was the primary site of action of picrotoxin in the frog. Eccles, Schmidt and Willis (1963) observed that picrotoxin caused a block of primary afferent depolarization but was ineffective on postsynaptic inhibition in the cuneate nucleus. Llinas (1964) confirmed this by demonstrating that inhibition of the monosynaptic reflex of quadriceps motoneurons in the cat was practically unaltered by picrotoxin at the stage at which presynaptic inhibition was almost completely blocked.

Boyd, Merritt and Gardner (1966) demonstrated that picrotoxin blocked presynaptic inhibition of cuneothalamic relay cells by studying the effects of this agent on the recovery cycle of the leminiscal response. Banna and Jabbur (1968; 1969), by testing the excitability of cuneate afferent terminals in the cat, similarly concluded that picrotoxin blocked presynaptic inhibition.

Shende and King (1967), using chloralosed squirrel monkeys, showed that picrotoxin, when administered intravenously, depressed cortically evoked primary afferent depolarization of the trigemminal brain stem nuclei, and Bannerjee, Feldberg and Georgiev (1970) found that the cerebral cortex was more sensitive to picrotoxin than to leptazol or strychnine and that spike discharges continued for longest after its administration: a few hours after picrotoxin; one hour after strychnine, and only for a few minutes after leptazol. Frank and Jhamandas (1970), using isolated slabs of cat cerebral cortex, reported that picrotoxin induced stimulant activity of long duration, consisting of large negative spikes followed by positive bursts and strong epileptiform after-discharge.

Hemsworth and Neal (1968) found that picrotoxin increased the release of acetylcholine from rat cerebral cortex to a much greater extent than other central stimulant drugs tested, which included strychnine, leptazol and caffeine. Hill, Simmonds and Straughan (1972) showed that picrotoxin antagonized the depressant effect of gamma-aminobutyric acid in feline cortex, and Engberg and Thaller (1970) that this drug selectively depressed the action of GABA on the spinal cord. Harris, Hopkin and Neal (1973) found that picrotoxin was the only convulsant studied which significantly inhibited the uptake of GABA by slices of cerebral cortex.

Bemegride: Bemegride does not block postsynaptic inhibition of

spinal neurons (Curtis, 1959). Banna and Jabbur (1970) noted that, in decerebrate and in anaesthetized cats, intravenous injections of bemegride reduced the increase in excitability of cuneate afferent terminals produced by conditioning cutaneous stimuli, and that the dorsal column reflex recorded from the superficial radial nerve after direct cuneate stimulation was completely blocked. They concluded that bemegride, like picrotoxin, blocked depolarization of presynaptic terminals of muscle and ascending cutaneous primary afferent fibres and thus reduced presynaptic inhibition. Frank and Jhamandas (1970) found that bemegride stimulated the cerebral cortex directly, increasing the amplitude of surface negative and surface positive responses and producing spiking during bursts of activity.

Two features in the pattern of bemegride-induced convulsions indicate that it might prove a useful tool in the investigation of the control of flexors and extensors. In the early stages, the flexor movements indicate that the slow fibres may be more active than the twitch fibres, by increase in tone rather than in the onset of twitches; secondly, the suppression of the extensor while the flexor is undergoing quite strong convulsions.

Insulin: Apart from the finding of Sutin (1963) that direct injection of insulin into the hypothalamic ventromedial nucleus of the cat was without effect on evoked potentials, and the observation of Sloviter and Yamada (1971) that insulin added to the perfusion fluid of the isolated brain or injected into rats from which brain preparations were subsequently made did not alter the spontaneous electrical activity, investigations of this hormone have been concerned with its metabolic effects. It is probable that an investigation of the action of insulin during convulsions would reveal more positive effects.

Ross (1952) studied the effect of insulin across the blood-aqueous barrier of the eye and (Ross, 1953) into the isolated lens, and found that the hormone increased glucose uptake by 350%. The view that the entrance of sugar into cells was governed by a transfer system, which promoted the rapid entrance of glucose as a free sugar from the extracellular fluid, was put forward by Goldstein, et al. (1953). This opinion was maintained by Stadie (1954) and, in a Croonian lecture, Young (1962) claimed that the idea of a single locus of action on permeability barriers was tenable. Dormandy and Zardy (1965), using erythrocytes, suggested that insulin led to a change in the oxidation-reduction potential gradient across the cell membrane. Weissberger (1945) observed that insulin, like adrenaline, caused a decrease in the amount of inorganic phosphate in blood. Sacks (1953) concluded that, in the diabetic animal, there was a diminished turnover of the labile phosphorus of adenosine triphosphate-adenosine diphosphate, glucose-1-phosphate, and glucose-6-phosphate. It is interesting to note that the acetylation of aromatic amines is dependent upon the availability of high-energy phosphate (Lipmann, 1945) so that a raised insulin level could conceivably result in an increased formation of acetylcholine.

Nelson, et al. (1968) found that the administration of insulin to alloxan diabetic animals, together with enough glucose to maintain high plasma levels, at least doubled the brain glycogen in 6 hours. They also noted that insulin could increase the ratio of brain-to-plasma glucose, an effect also found with barbiturates, the highest ratio being obtained by a combination of hormone and anaesthetic. Strang and Bachelard (1971) also observed a rise in brain glycogen 3 - 5 hours after administration of insulin to rats and concluded that insulin

probably slightly increased glucose transport from blood to brain, but at a rate which was within the capacity of the hexokinase to phosphorylate the increased glucose. Phelps (1972) suggested that fluctuations in brain glycogen probably occured primarily in astrocytes, but that astrocyte glycogen levels might be influenced by changes in neuronal activity.

Caffeine: In the frog, direct exposure to caffeine will cause contractures to develop in purely tetanic muscles, as well as in those containing slow fibres (Sommerkamp, 1928). Gasser (1930) pointed out that the caffeine contractures are slow in onset, but eventually develop considerable tension, and that high concentrations destroy the cross-striations and cause the contents of the fibres to collect in an amorphous clump, the muscles becoming hard and white and going into rigor. Ludin, Luttgau and Oerliker (1966) found that, in frog muscle, on exposure to caffeine (1 - 10mM), tension of full tetanus magnitude was reached with a half-time of 2 - 4 seconds, and complete relaxation occurred with a similar speed after removal of the drug. Chiarandini, et al. (1970) observed that crayfish muscle fibres differed from those of the frog, in that caffeine-induced contractures were transient. It seems pertinent to stress that, in the preparation used here, ligation of the thigh prevents injected caffeine from reaching the muscles and their contained motor nerve terminals. Nevertheless, experiments which have been carried out to investigate caffeine-induced contractures may throw light on the mechanism of action of the xanthine in the central nervous system.

Caffeine penetrates the cell membrane rapidly and enhances the efflux of calcium, probably by reducing calcium binding both in the

membrane and myoplasm (Bianchi, 1961; 1962). Since caffeine can induce contractures in muscles in a calcium-free solution at a time when potassium-induced contractures have been completely eliminated (Frank, 1960b) and in muscle fibres depolarized in media with high concentrations of potassium (Axelsson and Thesleff, 1958), Frank (1962) thought it was probable that the xanthine caused a contracture by releasing sufficient calcium from a binding site in or on the muscle to cause a significant increase in the intracellular levels of ionized calcium, and that these calcium ions initiated the mechanical response. Ludin, Luttgau and Oerliker (1966) favoured the idea that the drug acted at some part of the sarcotubular system, possibly the T-system, but Gruener (1967) has elicited contractures in stripped amphibian and mammalian fibres and concluded that intracellular calcium stores were necessary. Gebert (1968) suggested that, in high concentrations, caffeine not only liberated calcium from the bound form but also inhibited binding of the free calcium by the sarcoplasmic reticulum.

Hofman (1969) reported that miniature end-plate potentials incresed in frequency with caffeine, and suggested that this might be due to the liberation of free calcium in the nerve terminals facilitating the release of acetylcholine. Elmqvist and Feldman (1965) also noted a marked rise in m.e.p.ps when caffeine was applied in the presence of normal calcium-ion concentrations and an increase in rate even when calcium was absent from the surrounding fluid. Cochrane and Parsons (1972) found that caffeine increased the rate of postjunctional membrane repolarization following bulk applications of carbamylcholine in the presence of calcium, using sartorius muscles of the frog, and deduced that the drug increased the calcium permeability of the surface membrane. Cooke, Okomoto and Quastel (1973) have recently investigated the role of calcium in depolarization-secretion coupling at the motor nerve

terminal.

Ritchie (1970) states that the xanthines act as competitive inhibitors of phosphodiesterase, an enzyme which inactivates cyclic adenosine-3'-5'-monophosphate and that the resulting increased glycogenolysis might promote cellular processes normally induced by stimulation. Jankelson, et al. (1967) demonstrated that, in men with maturity-onset diabetes, the blood-glucose rose after coffee, the maximum being reached in 20 minutes, with return to normal in about one hour. There was no concomitant rise in serum-insulin. Sattin (1971) reported that pretreatment with caffeine prevented elevation of cyclic 3'-5'-AMP in the forebrain of the mouse during seizures induced by electroconvulsive shock. The drug did not alter the initial level of the nucleotide. Northrop and Parks (1964) found that pretreatment of intact rats with theophylline potentiated 3'-5'-AMP-induced hypoglycaemia.

Hemsworth and Neal (1968) found that caffeine produced no significant change in the release of acetylcholine from rat cerebral cortex. Frank and Jhamandas (1970) observed that local application of caffeine to isolated slabs of cat cerebral cortex increased the amplitude of surface negative and surface positive responses, but there was no spiking. Weirs and Laties (1962) have reviewed the enhancement of human performance by caffeine.

EFFECTS OF PRETREATMENT WITH ANTICONVULSANTS ON THE CONVULSIONS INDUCED BY STRYCHNINE, LEPTAZOL, AND BEMEGRIDE.

Just as the patterns of convulsion induced by various agents may throw light on the central control of flexors and extensors, so may the actions of anticonvulsants. Osuide (1968) has described the effects of mephenesin, chlorpromazine and orphenadrine on the convulsions induced by strychnine, leptazol, and picrotoxin, and the effects of these centrally-acting drugs on spinal reflexes in the chick. The modification of strychnine-induced convulsions by anticonvulsants in the frog has already been described (Bennett, 1972) and this Section is an extension of that work.

METHOD. The procedure was the same as in examining the patterns of convulsion (pp. 87 - 88), except that an anticonvulsant was administered, again by injection into the cranial dorsal lymph sac, at a known time, usually 15 minutes, prior to the injection of the convulsant.

Doses are given in the relevant Table with the results.

RESULTS:

Strychnine: These are included in the Paper referred to(Bennett, 1972) of which the results, Table and Figures are presented. For the purpose of this Thesis, the Table and Figures are assigned appropriate numbers.

RESULTS

Sixty-two frogs (*Rana temporaria*) were used in these experiments, the results of which are summarized in Table 1.

Short-term pretreatment ($\frac{1}{4}$–$2\frac{1}{2}$ hr) with reserpine delayed the onset and markedly reduced the duration of the maximum sustained convulsion (Fig. 1). Prolonged pretreatment (4–96 hr) had less effect on the start but increased the duration of the maximum sustained convulsion beyond the normal (Fig. 2). A consistent relation between dose and effect was obtained in each condition. The mean maximal plateau tensions developed by the extensor and flexor were tabulated but were necessarily less reliable and, in the main, proved statistically insignificant.

TABLE 1. EFFECT OF PRETREATMENT WITH ANTICONVULSANTS ON STRYCHNINE-INDUCED CONVULSIONS IN THE FROG (*Rana temporaria*)

Drug	Dose (μg)	Period of pretreatment (min)	No. of animals	Longest sustained convulsion		Maximum tension developed	
				Start (min)	Duration (sec)	Extensor (g)	Flexor (g)
Control	—	—	20	4·0±0·3	63·6± 6·3	13·9±2·2	7·3±1·5
Reserpine	60	150	2	6·7±2·3	20·0±12·0†	20·0±5·0	13·0±9·0
Reserpine	250	15	4	14·5±4·9*	5·5± 1·9†	6·8±2·0*	3·0±1·7
Reserpine	500	15	2	5·6±1·7	2·5± 0·5†	11·0±4·0	6·0±4·0
Reserpine	60	4–96 hr	8	5·8±0·8*	53·8± 8·8	11·6±2·8	7·4±1·4
Reserpine	250	4–48 hr	6	6·7±1·7	145·0±22·6†	9·5±2·1	6·2±0·7
Chlorpromazine	150	10–30	6	7·0±1·9	38·7± 6·1†	9·0±2·2	5·0±1·8
Mephenesin	100	15	4	5·1±0·6	31·5± 5·4†	9·8±2·8	5·3±0·8
Mephenesin	500	15	4	19·3±8·7	11·8± 3·5†	7·3±2·1*	2·7±1·2*
Orphenadrine	15	20	2	4·3±0·6	23·0±12·0†	2·8±2·6†	2·6±2·5
Orphenadrine	100	15	4	8·1±1·0†	10·0± 2·7†	3·0±0·9†	2·0±0·4†

Results expressed as mean ± S.E. Significance of difference from control $*P < 0{\cdot}05$; † $< 0{\cdot}005$. This demonstrates that the duration of the most prolonged convulsion is a suitable parameter where the one insignificant difference may be attributed to depletion just offsetting the release of amines by reserpine.

Chlorpromazine, mephenesin and orphenadrine all delayed the onset and decreased the duration of the maximum sustained convulsion. At a dose of 0·1 mg, orphenadrine proved the most effective in both respects. The tensions developed, in general, proved consistent with dosage but of poor significance. Orphenadrine, given alone, was found to produce tremors in about 35 min and weak convulsions in about 60 min after subcutaneous injection.

Table 18: Effect of pretreatment with anticonvulsants on strychnine-induced convulsions in the frog (Rana temporaria). (Table 1, above).

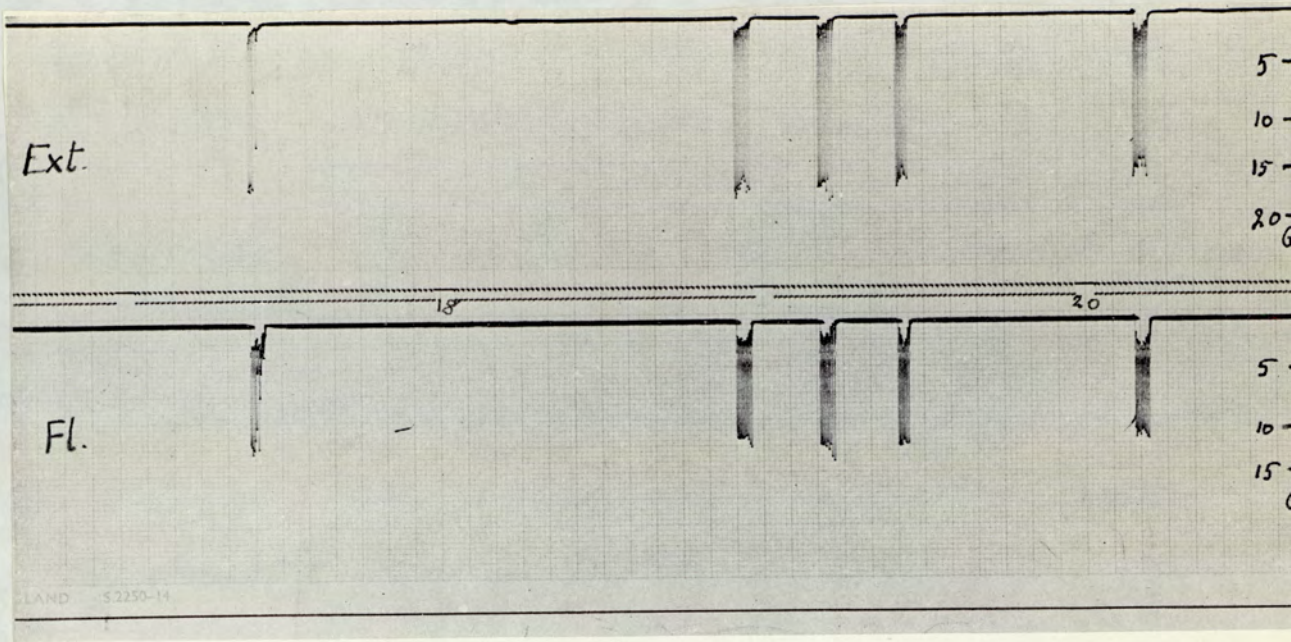


FIG. 1. Effect of amine-release on strychnine-induced convulsions in the partly decerebrate frog. Records taken from left peronaeus longus (upper trace, Ext.) and left tibialis antichus lower trace, Fl.) using 220-g strain-gauge transducers. Time base, 1 min. Tension scale, grams. 0·5 mg reserpine at time 0; 1 mg strychnine at 15 min.

Fig. 30. Effect of amine release on strychnine-induced convulsions.

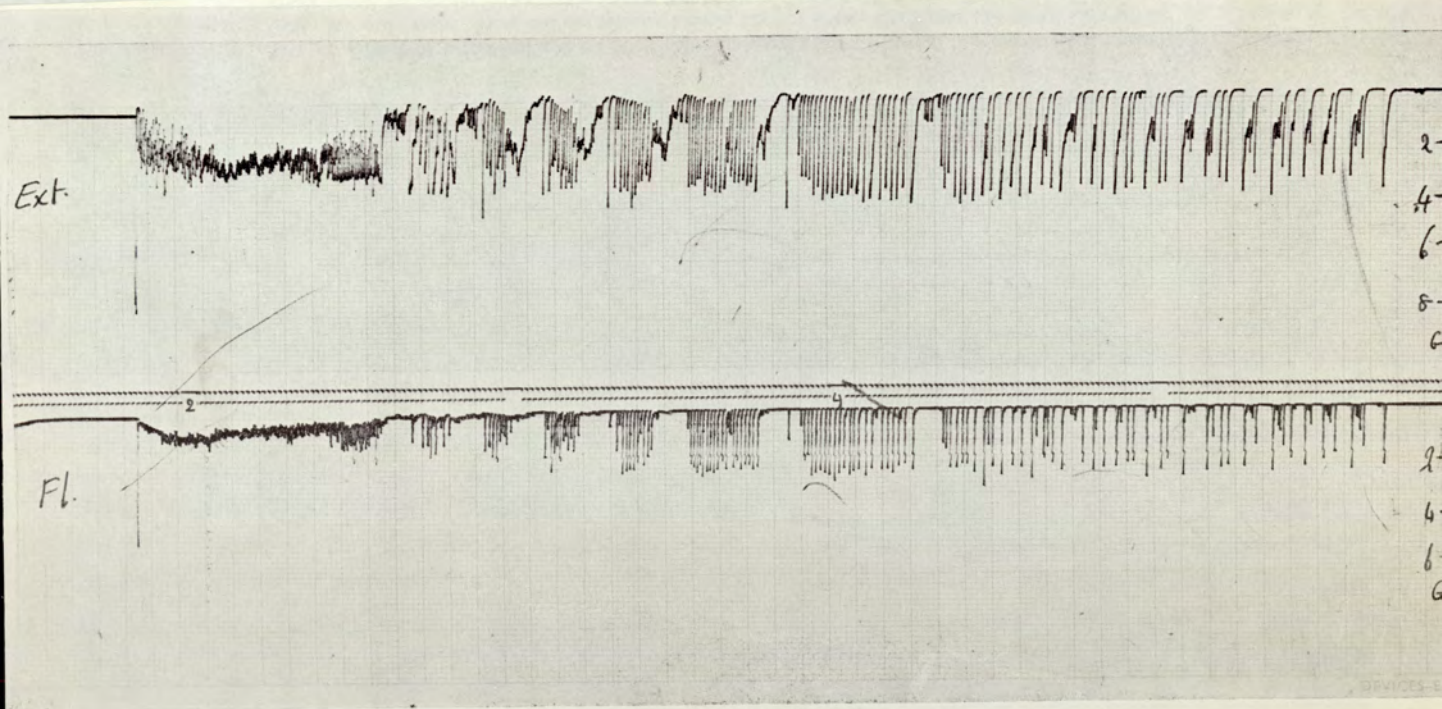


FIG. 2. Effect of amine-depletion on strychnine-induced convulsions in the partly decerebrate frog. Records, as in Fig. 1. Pretreated with reserpine (0·25 mg daily) for 48 hr; 1 mg strychnine at time 0.

Fig. 31. Effect of amine depletion on strychnin-induced convulsions.

Bemegride: The results for bemegride are summarized in Table 19. Short-term pretreatment with reserpine, thereby releasing catecholamines, significantly shortened the duration of the longest sustained convulsion, using doses of 0·13 and 0·25mg. The time of onset was significantly delayed with the higher dose. Chlorpromazine, in a dose of 0·5mg., significantly hastened the onset of the longest sustained convulsion of the flexor but had no other significant action. Mephenesin, again, hastened the onset of the longest sustained convulsion of the flexor, but it significantly reduced the duration of this convulsion in the extensor. Orphenadrine, in a dose of 0·1mg., significantly hastened the onset of the longest sustained convulsion and shortened the duration of this convulsion, perticularly that of the extensor.

Leptazol: The available results for leptazol are summarized in Table 20. The only significant result was a delay in the onset of convulsions, both of flexor and extensor, produced by pretreatment with orphenadrine.

DISCUSSION: Short-term pretreatment with reserpine, which may be assumed to flood the brain with catecholamines, caused the convulsions induced by strychnine to be discontinuous from the start; the onset was delayed and the duration of the longest sustained convulsion significantly reduced. In the case of bemegride, similar pretreatment delayed the onset of convulsions to a greater extent, and shortened the duration of the longest sustained convulsion to a lesser extent, than in the case of strychnine; the ability of the extensor to maintain a convulsion was more affected than that of the flexor. In the two experiments in which reserpine was administered prior to leptazol, the onset of convulsions was delayed and the longest sustained

Dose A-C.	Time Pre.	No. An.	Longest sustained convulsion			
			Flexor		Extensor	
			Start	Duration	Start	Duration
mg	min		min	sec	min	sec
Controls						
		9	23.3± 4.1	114.2±24.2	24.3± 4.1	70.7±15.8
Reserpine						
.025	15	2	17.5± 8.5	130.0±10.0	17.5± 8.5	100.0±20.0
.13	10	4	46.8±12.6	11.5± 2.2**	48.5±12.1*	6.8± 1.9**
.25	15	9	44.6± 4.8**	15.3± 3.6**	49.1± 5.3**	7.4± 0.7**
Chlorpromazine						
0.1	15	7	20.7± 5.9	95.5±15.7	19.4± 5.2	86.7±24.0
0.5	15	6	9.3± 2.3**	92.2±29.4	15.2± 7.2	37.2±18.6
Mephenesin						
0.1	15	4	13.8± 3.4*	127.3±33.3	22.3± 1.6	20.1±13.6*
0.5	15	6	19.8±10.3	122.3±40.3	26.3±14.0	13.0± 4.2**
Orphenadrine						
.015	15	3	28.3±10.7	105.7±53.4	28.3±10.8	82.0±38.9
0.1	15	6	12.2± 3.7*	74.3±70.0	12.3± 3.7*	16.5± 8.7**

Table 19. Effect of pretreatment with anticonvulsants on bemegride-induced convulsions in the frog (Rana temporaria). 1mg. bemegride injected subcutaneously. Results expressed as mean ± S.E.;

** $P < 0.005$; * $P < 0.05$; Dose A-C = dose of anticonvulsant; Time Pre. = time of pretreatment with anticonvulsant; No. An. = number of animals

* Significance of difference from controls:

Dose A-C	Time Pre.	No. An.	Longest sustained convulsion: Flexor Start	Flexor Duration	Extensor Start	Extensor Duration
mg.	min.		min	sec	min	sec
Controls						
(1 - 3)		5	13.2±5.5	26.8±11.3	14.0±5.6	16.0±8.7
(4 - 5)		11	17.3±4.8	80.5±18.8	17.8±4.8	33.2±8.5
(8 - 10)		6	7.2±2.2	102.3±31.1	8.3±2.9	69.0±24.3
Reserpine						
.25	15	2	27.0±24.0	14.5±5.5	27.0±24.0	14.5±5.5
Chlorpromazine						
0.1	20	3	18.0±1.2	18.5±11.5	18.5±0.5	21.3±7.7
Mephenesin						
0.1	10	1	26	11	26	7
Orphenadrine						
.015	3	5	33.6±3.3*	47.2±23.4	33.6±3.3*	32.2±17.3

Table 20. Effect of pretreatment with anticonvulsants on leptazol-induced convulsions in the frog (Rana temporaria). In tests, 3mg. leptazol injected subcutaneously. Results expressed as mean ± S.E.; Dose A-C = dose of anticonvulsant (in controls varying doses of convulsant, in brackets); Time Pre. = time of pretreatment with anticonvulsant; No. An. = number of animals. Significance of difference from controls: * $P < 0.05$.

convulsion was of equal duration in the two muscles; since, in the c controls, the flexor had sustained the convulsion the longer, it may be that the centres controlling it are more susceptible to catecholamines. Chen, Ensor and Bohner (1954) found that reserpine did not alter the strychnine-induced seizure pattern, nor the dose required for maximal tonic extensor response in the mouse and that, under reserpine, leptazol produced a seizure pattern similar to that produced by strychnine and that reserpine lowered the convulsive seizure threshold to leptazol.

Chlorpromazine delayed the onset of convulsions induced by strychnine and leptazol, but hastened the onset of those induced by bemegride, more particularly those of the flexor. Chlorpromazine significantly shortened the convulsions induced by strychnine, but no significant effect was produced on the duration of those induced by bemegride or leptazol. Klerman and Cole (1965) reported that chlorpromazine did not alter the various components of convulsions due to leptazol in rabbits. Osuide (1968) reported that with minimal effective doses of chlorpromazine the flexor, crossed extensor, and patellar reflexes were all augmented, though the flexor reflex initially underwent a transient depression. He found that this agent did not affect the augmentation of the reflex contractions produced by strychnine or leptazol, or protect chicks against lethal doses of these convulsants. Available evidence indicates that chlorpromazine depresses motor function mainly by action on the reticular formation of the brainstem (Lloyd, 1941) and spinal cord (Hudson, 1966). Guth and Spirtes (1964) suggested that the antipsychotic property of chlorpromazine was the result of a modification of membrane permeability through inhibition of transport adenosine triphosphatase, but Ebadi and Carver (1970) found that depression and stimulation did not necessarily alter ATPase. Andèn,

et al. (1967) produced evidence that chlorpromazine caused an increase in the activity of the dopamine and noradrenaline neurones in the central nervous system.

Mephenesin: Mephenesin delayed the start and reduced the duration of the convulsions induced by strychnine and leptazol. Administered prior to bemegride, this centrally-acting muscle relaxant hastened the start but did not significantly alter the duration of convulsions in the fexor, and did not affect the onset but reduced the duration of those of the extensor. Osuide (1968) found that mephenesin blocked the flexor, crossed extensor, and patellar reflexes in the chick, but that in the cat the patellar reflex was resistant to this agent. He also found that mephenesin antagonized the augmenting actions of strychnine and leptazol on these reflexes and protected chicks against lethal doses of leptazol, but not of strychnine. Mephenesin was the most potent muscle relaxant of a series of glycerol esters studied by Berger and Bradley (1946) and is thought to inhibit activity in motoneurons. Boyd, Merritt and Gardner (1966) ascertained that when mephenesin was administered in conjunction with strychnine a second postsynaptic inhibition of the relay cells was blocked.

Orphenadrine: Orphenadrine alone is capable of inducing tremors and weak convulsions in the frog. Osuide (1968) similarly found that this agent caused tremors and convulsions in the chick. The doses used in pretreatment were subconvulsive. Prior to strychnine, orphenadrine delayed the onset and shortened the duration of convulsions. Prior to bemegride, it hastened the start but again reduced the duration of convulsions, more markedly those of the extensors. It delayed

the onset of leptazol-induced convulsions without significant effect on their duration. In minimal effective doses, Osúide (1968) found that orphenadrine augmented the flexor, crossed extensor and patellar reflexes but that this was changed to depression by serial injections of large doses. He found that orphenadrine did not antagonise the stimulant effects of strychnine or leptazol, but in large doses potentiated strychnine; it did not protect chicks against lethal doses of either strychnine or leptazol. Orphenadrine may inhibit a preferential uptake mechanism for noradrenaline (Bassett, Story and Cairncross, 1968). Roozemond and Nauta (1966) found that orphenadrine significantly raised the level of bound serotonin in rat brain, the effect being maximal in one hour after administration.

CONCLUSION

Sherrington (1904, 1906) described the scratch, extensor thrust, crossed extensor, and flexion reflexes in the dog and the removal by strychnine (Sherrington, 1907) of reflex inhibition in skeletal muscles. He continued this work by discussing the integration of reflexes in the activities of stepping and standing (Sherrington, 1910, 1913). With Liddell, he characterized the d'emblée and recruit type reflexes (Liddell and Sherrington, 1923, a, b, c). The present study has sought to shed further light on adaptations to the functions of flexion and extension.

In the shank of the frog, it has been shown that the tibialis anticus, a flexor, is heterogenous and that the peronaeus longus, an extensor, is homogenous. It was known that the gastrocnemius, which is a flexor of the knee and an extensor of the ankle is mainly homogenous but contains two heterogenous tonus bundles. The heterogenous nature of the flexor accords with the fact that it withdraws the limb rapidly and maintains the withdrawal, whereas the homogenous extensor exerts a simple thrust. The muscle fibres responsible for the withdrawal of the flexor and for the thrust of the extensor have, hitherto, been jointly described as twitch (fast), the fibres responsible for maintaining the withdrawal of the flexor are tonic (slow). The structure and properties of tonic (slow) muscle fibres have been defined by other workers, here evidence is adduced that the twitch fibres of a flexor are fast compared with those of an extensor.

Optical microscopy revealed that twitch fibres of the flexor were of smaller diameter than those of the extensor. Under the electron microscope, differences in various parameters of the myofibrils of twitch fibres from tibialis anticus and peronaeus longus were

observed.

A study of the concentration relations of potassium and sodium indicated that the resting potential of the twitch fibres of tibialis anticus was higher than that of peronaeus longus. This could assist rapid flexion.

The electrophysiological data indicated that the flexor had the shorter latent period, more rapid shortening, slightly the higher fusion frequency, and the greater capacity to maintain a tetanus, while the extensor developed the greater tension in a twitch. All these features are consistent with functional requirements.

A study of the available literature indicated that it was generally true that, in the hindlimbs of amphibians, a flexor is heterogenous, an extensor homogenous, while a muscle which acts on two joints is mainly homogenous but contains one or more tonus bundles. In the forelimbs, anomalies may arise from the rotation involved in the adoption of the plantigrade posture. In digits, the difference between dorsiflexion and plantar flexion may not call for the same degree of differentiation.

In mammals, likewise, it is found that a flexor is heterogenous and an extensor homogenous. In this case, the fibres responsible for the withdrawal of the limb are white (fast), while those responsible for the thrust of the extensor are red (slow). The fibres responsible for the maintenance of the withdrawal of the limb by the flexor are red. No distinction has been made between red fibres in the flexor and extensor of a mammal to date. Nothing analogous to the tonus bundle has been described in mammals. Here, an attempt has been made to draw and confirm analogies between the muscle-fibre types in amphibians and mammals.

The use of strychnine as a neuromuscular blocking agent of the

competitive type demonstrated that the twitch fibres of the flexor were less sensitive than those of the extensor, confirming their respective analogy to white and red fibres in the mammal. There was some indication that the sensitivities to suxethonium were in agreement with this conclusion, in that the initial potentiation of the twitch fibres of tibialis anticus tended to be greater than that of those of peronaeus longus. Neuromuscular blocking agents of the depolarizing type were also employed to assess the proportions of slow fibres in the gastrocnemius of frogs and toads. Toads, which walk, have a higher proportion of slow fibres than frogs, which leap.

In the isolated organ bath, excess of potassium caused contractures in both the flexor and extensor, the former proving the more sensitive. On washing out, the flexor tended to maintain the contracture, the extensor to relax to a greater extent than prior to the addition of the salt.

In muscle, there are other features which could well be involved in the adaptation to flexion and extension. There have been few investigations of the muscle spindles in a flexor, but Granit, Pompeiano and Waltman (1959, a, b) have indicated differences between those of a flexor and extensor of the cat and Rutledge and Haase (1961) have adduced evidence that the muscle spindles of a flexor contain a slow tonic and a fast phasic motor mechanism, and that the latter is activated before the first indication of a change in muscle tension. Attention has been drawn to the fact that Hess (1970) found that twitch fibres associated with slow fibres in the frog had nerve-endings which were shorter, more branched, and more variable in appearance than the typical Endbuschel of an homogenous muscle.

The various patterns of convulsion induced by particular agents indicate differential action on centres controlling and co-ordinating

flexors and extensors. Drugs which antagonise or potentiate convulsions may also exert a differential action. Callingham and Sharman (1970) have determined the concentration of catecholamines in gross regions of the brain of the domestic fowl. Progress on these lines would be facilitated by the application of a method which would enable the location and estimation of synaptic transmitters in more limited areas. The technique of free-zone electrophoresis (Hjerten, 1970) could well prove suitable and has the advantage of eliminating the need for de-fatting. The location of sites where the release or uptake of specific transmitter substances took place under the influence of stimulants and depressants of the central nervous system would be of great value. Such studies would obviously be simpler in higher animals than the frog, with larger and more developed brains. The same holds true for investigating the electrical effects of drugs acting on excitatory and inhibitory synapses. A full assessment of the results of experiments with centrally-acting drugs must await such developments.

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