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Despite the continuous efforts made during the past 30 years in muscle physiology, the extent of our knowledge in some parts of this field still remains comparatively slight. This is first and foremost due to the fact that as a rule the muscle has been considered experimentally as a structural unit, no attention having been paid to the many different elements of which it is composed, which may vary widely in their physical, chemical and physiological properties. Furthermore it has not been generally realized that the shape of the contractile elements themselves and their arrangment in the muscle will influence profoundly the mechanical behaviour of the tissue. Moreover, on the basis of experiments using whole muscles attempts have been made to draw conclusions concerning the phenomena taking place in the microscopic and ultramicroscopic parts of the muscular fibril. Thermal investigations also have met with difficulties arising from such causes; it has for instance been found impossible to secure a uniform temperature throughout the whole of the preparation, resulting in considerable uncertainty in assessing experimental evidence; and the importance of the shape of the fibres has not been appreciated. Investigations of the electrical properties of muscles have not been performed with regard to such considerations either. For instance the result of measurements of resistance in a whole muscle is of limited value, because we do not know the paths which the current will follow in the tissue. If it

is desired to study the electrical properties of the fundamental contractile mechanism, then the experimental object must be the single muscle fibre; because in the whole muscle such factors as connective tissue, blood vessels of varying calibre etc., will interfere considerably with the measurements.

There are, of course, problems which can be solved through experiments on whole muscles and some again which can only be solved by the use of such preparations. But in most cases this method of approach is ruled out, because the very numerous sources of error arising from the highly complicated structure of the muscle can neither be eliminated nor properly controlled. Therefore, more and more use has gradually been made of the functional unit — the single muscle fibre. The definition thus implied may be criticised on the grounds: firstly that even in muscles allowing the most finely graded movements, more fibres are present than axis cylinders in the corresponding motor nerve; so that a muscle fibre cannot come into action singly; secondly, recent electrostatic measurements show that the muscle is composed of a vast number of relatively independent subdivisions (sarcomeres); yet nevertheless, it must for the time being, be considered permissible to regard the fibre as the functional unit under physiological-conditions, as in such circumstances a partial contraction of a fibre is unknown. Since the muscle fibre is thus neither structurally nor functionally considered to be the ultimate unit, the value may be questioned of employing these small objects, in many respects so difficult to handle; the more so since the isolated fibre, despite all possible care, has a very limited life as an experimental object.

It is, of course, for such reasons that where experimental

conditions have permitted, bundles containing a small number of fibres have been used. But greater difficulties are met with in the whole muscle. Even pairs of symmetrically situated muscles in the same animal differ in structure, so that the variations in the results of experiments with whole muscles will in many cases become so great that it becomes possible only to discuss the functions of the muscle in approximate terms. Therefore, when the essential features of muscular function had to be investigated attempts have always been made to infer from such results the behaviour of the single fibre. This has, however, often proved to be impossible, not only because the experimental errors may become of a higher order of magnitude than the reaction which it is desired to study, but also because variations are so great as to require an impracticable number of experiments before reliable results can be obtained by the use of statistical methods. By working with the single muscle fibre we eliminate a number of the major sources of error, the conditions of the experiments become far simpler, and unavoidable errors become of more reasonable size in proportion to the physiological reactions. This method of approach, during the few years in which it has been used, has proved fruitful and has thrown new light on previous investigations, which may be said to justify its introduction.

Anatomy of the Striated Muscle Fibre.

On the basis of the general concept of the organic kingdom that structure and function are intimately related, it would be appropriate to begin every account of the function of an organ with a discussion of its structure.

The anatomical investigations to be dealt with here are limited to the skeletal muscles of the vertebrates, especially of the frog, chiefly because the great majority of our own physiological experiments were performed with frog's muscles. We have, however, also experimented with lizard's muscles, especially so where the motor endplates were involved in the experiments.

Shape and Size of Muscle Fibres.

In order to determine the shape and size of the muscle fibre, it must be completely isolated. Within the bundles the fibres are often interwoven to such a degree that it becomes impossible to trace any one fibre for a considerable distance, especially towards its terminal portion, which is often extremely slender (Fig. 1.). As the fibres moreover are often overlapped in part by others, or deformed through pressure by adjacent fibres it will almost always be impossible without complete isolation to measure their thickness with requisite accuracy. When isolating the fibres, the bi-refringence method gives valuable information as to whether one or more of these is being dealt with, and has the particular advantage that the decision can be made without touching the fibre with any instrument.

If the isolated fibre has to be employed in physiological experiments there is only one method of isolation possible, viz. dissection. If, on the other hand, we only want to measure the shape and dimensions of the fibre, other methods may also be employed; but as not all those suggested seem to be reliable, only a few will be referred to here.

In Schwalbe's laboratory Mayeda (1890) employed the following procedure: He immersed the live animal in 20

per cent. nitric acid and then placed it in an incubator at 40° (C) for 24 hours. The preparation was then removed and carefully rinsed with water. Examination had to be made immediately after this preparation was completed. The advantage of this procedure appears to be that the animal whilst alive would have to swallow some of the fluid in which it was immersed, so that the action of the latter would presumably be more effective and more rapid than by penetration through the skin alone. This method, though unattractive, may be expected to give reliable results.

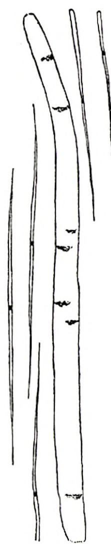

Fig. 1. Muscle bundle with the conical and spindle shaped fibres of which it is composed. Entrance of nerve twigs marked. (BARDEEN).

Another method, given by F. C. C. HANSEN (see LINDHARD (1926)), consists in boiling the muscle for 2 hours in a quantity of water that will just cover it, after which the boiled muscle is clarified in glycerine. The following modification of this method has been employed by LINDHARD. The whole of a frog's leg was placed in a beaker filled with ordinary tap-water, and covered by a glass-plate. When the water is heated to boiling, whether slowly or rapidly, a sudden extension of all the joints of the limb will occur at a certain temperature, and during the subsequent boiling the limb will remain in the same position. When boiling is finished and the skin cut off, the muscles can be removed. Muscles prepared in this manner have the same shape as fresh muscles but are slightly smaller in all dimensions. As a rule there are no signs of contracture, but if a single muscle is treated in the same manner it will be found after boiling in a state of maximal contracture.

The individual fibres may now be isolated with comparative ease under the low power binocular microscope. After it has been ascertained, using the high power, that the sarcolemma is uninjured, the fibre is then measured with the aid of an ocular micrometer.

It appears from such measurements and from similar observations on uninjured living fibres (see later), that the muscle fibres vary greatly both as regards dimensions and shape, and not only do such variations occur between different species but also between different muscles in the same animal. Great variations may even be found between fibres of the same muscle.

In the majority of the cases examined the muscle fibre is shorter than the bundle, sometimes much shorter, but there are also muscles in which the fibres run from one terminal tendon to the other. Most text-books appear to give rather superficial statements. However, Heidenhain's statement (1911) that only in muscles more than 12 cm. long are fibres found terminating within the bundle, whilst in all shorter muscles the fibres run from tendon to tendon, is probably incorrect. Moreover, his observation that the thickness is from 9—60 μ does not cover the whole range of variations.

Schäfer's (1893) statements are more accurate, saying that the length (with the exception of the sartorious) rarely exceeds $1^1/_2$ inch., whilst the thickness is 0.1—0.01 mm. — Mayeda (l. c.) examined the thickness of the fibres in the muscles of different animals throughout the whole range of vertebrates; but unfortunately he gives one measurement only, which makes it impossible to determine the shape of the fibres. In only a few cases was the length measured. These measurements show that the minimal thickness of

the fibres is approximately the same in all vertebrates, whilst the maximal thickness varies, decreasing in the following order:— Fishes, toads, reptiles, mammals, birds. The two latter classes are, however, only represented by one species each. The thickest fibres in a single individual muscle were found in the gastrocnemius, where this muscle occurred, and the thinnest in the ocular muscles.

In one case MAYEDA measured the length as well as the thickness of 48 fibres from a 26 mm. long sartorius of the R. esculenta, (giving, however, only one measurement of thickness). His results appear in Table I.

Table I.

	2	12	11	24	18	17	20
	2	14	24	11	24	24	23
	8	12	16	21	10	13	16
	9	4	16	19	22	16	25
	11	18	19	6	11	20	20
	7	11	7	11	22	22	..
	..	..	24	10	24	25	..
	..	..	15	..	13	25	..
Length, mm.	6.5	12	16.5	14.5	18	20	21
Thickness, μ	34—42	49—53	57—61	68—89	91—106	114—118	122—141

It will appear from the table that there is an approximate relation between length and thickness, the thickness increasing with increasing length. The average length of the 48 fibres is 15.7 mm., which shows that two fibres originating from opposite ends of the muscle overlap for a length of 5.4 mm.—

BARDEEN (1903) has given an excellent description of the structure of the obliquus abdominis externus in a number of mammals. Figure (1) represents a bundle consisting of three "fibre lengths", the centre one of which is composed of elongated spindle-shaped fibres, the tapering ends of which both end within the bundle.

Lindhard (1926) has measured the dimensions of the fibres of the gastrocnemius and the sartorius in the two experimental animals usually employed, namely Rana esculenta and R. temporaria. The gastrocnemius is composed of fibres of unequal length, the longest being those in the centre of the muscle. The average length of 10 fibres from a muscle 30 mm. long, was 4.6 mm.; the distal fibres are the shortest, hardly more than 1—1.5 mm. in length. In R. esculenta the fibres are bluntly conical. The ratio between the thick and the thin end of the fibre was 5.2:3, this being the mean of 18 measurements. In R. temporaria most of the fibres were irregularly cylindrical and arranged in pairs, a thick and a thin fibre alongside each other, the following measurements show:—

	I	II	III
Length in mm.	3.1—2.8	3.1—2.8	3.1—2.9
Thickness in μ	122—52	113—52	116—34

In the gastrocnemius the fibre thus runs from one terminal tendon to the other; but as a rule this is not so in the M. sartorius, especially not that of R. esculenta. The following figures are illustrative of the M. sartorius (R. esculenta):—

Muscle (longest fascicle)	27.9 mm.
Average length of fascicles	25.5 -
Average length of 117 fibres	17.2 -

The fibres will thus overlap each other for 9 mm., on the average. The ratio between length and thickness is best illustrated when the thickness of the fibres is measured in two places near the ends of the fibres:—

Length in mm.	13.5	14.8	25.5	15.1
Thickness in μ	9—104	46—122	9—101	49—107
Length in mm.	18.2	13.1	14.4	21.5
Thickness in μ	9—61	21—80	46—168	24—128

In a sartorius, 26 mm. long, the following fibre lengths were found:

5.2 7.6 8.6 11.5 12.4 13.9 16.2 19.6 22.0 24.0 mm.

In the sartorius of temporaria the average fibre length is greater in proportion to the total length of the muscle; the fibres frequently pass through the entire length of the muscle, but are rarely cylindrical.

From what has been stated above it seems highly improbable that any part of the sartorius is devoid of nerve fibres. This is shown by Kühne's early investigations (1860) and is borne out by Schimert's more recent publication (1934). Péterfi and Buchthal in unpublished experiments, have recently demonstrated with the aid of vital staining, fine nerve twigs in the pelvic end of sartorius.

Despite the considerable variation in shape of the muscle fibres, and the absence of any systematic investigations on this point, it is, however, possible to establish certain general types.

The muscle fibre is sometimes cylindrical; such fibres occur in various muscles of different species of animals, and are doubtless always comparatively short. The same applies to the bluntly conical shape described above in the gastrocnemius of R. esculenta. Long muscle fibres, on the other hand, are flagelliform (F. C. C. Hansen) or lanceolate and are then connected with the terminal tendons by their thick, rounded end whilst the thin "tail" is lost in the endomysium. Moreover Mayeda's work as well as that of Bardeen's and Lindhard's suggests that in certain muscles the fibres are shorter than the bundle, often considerably so, but on the average their length is more than half the length of the bundle, so that the thin fibre-ends overlap

for some distance. As fibres in the same muscle vary considerably in length this arrangement does not give rise to the occurrence of mechanically weak parts in the belly of the muscle. An additional mechanical support is provided by the occurrence in the muscle of varying numbers of elongated spindle-shaped fibres, i. e. fibres which are thickest in the centre and have long pointed ends, both of which are lost in the endomysium. The M- and N-fibres mentioned by Schiefferdecker (1902) are probably to be considered as artefacts, as the illustrations in his paper seem to indicate. It is often stated that in the tongue or the basihyoid membrane of the frog there may be found muscular cells showing tree-like branching. This statement, too, must at present be regarded with reserve. (Cf. Nogamitu (1931) cit. Rona's Berichte 65, p. 215). The isolation of muscle fibres is often so difficult, especially when they are very thin, that only with the utmost care and proper technique will it be possible to avoid errors such as those indicated here.

The fact that the muscle fibres are rarely cylindrical structures running parallel to each other from one terminal tendon to the other, ultimately "passing into tendinous fibres", makes it difficult to realise the exact nature of muscular contraction, or more precisely the process of relaxation. A muscle fibre can contract actively, but it cannot relax actively; so that if a fibre terminating in the endomysium contracts to, say, half its resting length, it will not be able to revert to its original length without the assistance of some external force. Häggquist (1920) has tried to overcome this difficulty. On the basis of his observations, he maintains, in agreement with Péterfi, that muscle fibres never pass directly into tendinous fibres. Neither does

he consider it possible that muscle and tendon fibres could be connected together by any cementing substance. In contradiction to W. J. Schmidt (1937) and Lubosch (1937) Häggquist, therefore, considers the terminal tendons to be continuous with the connective tissue stroma of the muscle, in the meshes of which the contractile substance is embedded and fixed by means of the Z-membranes (see later). In this manner the muscle fibres become closely connected with the surrounding tissues. One consequence of this conception is, that in any stable condition there exists in the muscle an equilibrium between the connective tissue stroma of the muscle and the active and inactive contractile elements, and that any transition from one stable state to another must be characterized by a disturbance of this equilibrium. Therefore comparisons of length and tension can only be made between muscles which have attained such stable conditions.

The functional significance of the shape of the muscular fibres is not quite clear, but it is not difficult to realise that the more or less pronounced conical shape, in particular, may give rise to considerable experimental difficulties, especially in connection with myothermal investigations.

Microscopic Structure of the Fibre.

Evaluation of the very comprehensive histological literature that exists concerning the muscle fibre is beset with great difficulties, because the great majority of the observations have been made on fixed and stained, i. e. dead material, and only in very few cases have attempts been made to verify the results so obtained by examination of living fibres. It is notorious that fixatives frequently change the tissues to a great extent, so that it frequently becomes im-

possible to decide whether the structure seen in the stained preparation is a picture of the structure preexisting in the living cell, or whether it is an artefact. In such work it is difficult, if not impossible, to safeguard against systematic errors.

It may be unwise to draw conclusions as to function only from observations on cadaver material; and it is an especial task of the physiologist to estimate the value of such work, in its applications to his own problems.

Microscopic examination of the muscle fibre shows that it is enclosed in a membrane, the sarcolemma, consisting of fine fibrils arranged in a network, the meshes of which are filled by a homogeneous substance (Péterfi).

Immediately beneath the sarcolemma are found the nuclei of the muscle cells, the number of which varies, but at any rate is very large. The nuclei are oval and are surrounded by a small amount of granular cytoplasm located especially towards the ends of the nucleus. During development the nuclei are at first situated throughout the fibre substance, but as the contractile elements proper are developed, the nuclei move towards the periphery of the cell. In the cells of frog's muscle, however, a few nuclei are to be found in the fully developed muscle fibres scattered irregularly throughout the fibre substance. The fibre is composed of an apparently undifferentiated substance, the sarcoplasm, in which a number of highly differentiated fibrillar structures, the myofibrils, are deposited. The deviation of the interference stripe, which indicates the degree of bi-refringence (see Fig. 9) shows also the presence of numerous spikes, pointing to the existence of fibrillar structure. The myofibrils occur either more or less uniformly scattered throughout the sarcoplasm or united in

bundles of various sizes (sarcostyles), which appear in transverse section of the fibre as irregularly polygonal figures (COHNHEIM's areas). Two types of muscle fibre are distinguished, according to the proportion existing in them between sarcoplasm and fibrils. In one of them, the "red" fibres, there is a relatively large amount of sarcoplasm, whose content of myoglobin determines the red colour, whilst in the other type, the "pale" fibres, there is only quite a small amount of coloured sarcoplasm.

In the majority of muscles of vertebrates a mixture of the two types is found; but in certain cases the whole of a muscle is formed by one or other type of fibres, forming respectively "red" and "pale" muscles. Various workers claim to have shown that the two types of fibres are responsible for differences in the behaviour of the muscle, pale muscles being said to contract more quickly but also to fatigue sooner than the red. It seems, however, as if the differences pointed out may all have their explanation in the varying content of myoglobin in the two types of fibre. As far as is known, no differences have so far been shown between the contractile elements proper, the myofibrils, in red and pale fibres and, therefore, these questions will not be treated further here.

Under the microscope the myofibrils prove to consist of two substances forming regularly alternating discs of different height. One of these substances is clearly doubly-refractive, whilst the other as a rule has been considered to be singly refractive; however, according to W. J. SCHMIDT this substance also shows a slight degree of double refraction. As the isotropic and anisotropic layers of the fibril are lying at the same level in all the fibrils, the fibre as a whole thus becomes "striated". The border between the isotropic and

the anisotropic layers is not sharp but still distinctly recognizable. When the muscle fibre is treated with KOH of suitable strength it falls apart into discs (Bowman's discs). If, in fibres isolated according to the method of boiling referred to previously, the sarcolemma is torn, it will be possible to isolate the fibrils, at any rate for short distances. Various workers have observed and described numerous details in the microscopic picture of the muscle fibre. For example Krause has demonstrated that corresponding to the middle of the isotropic layers there is a fine dark line (the "Z-Membrane"), which was considered by him to be a membrane. This finding has been accepted by most subsequent workers, and in the histological literature references are generally made to "Krause's membrane", or the basal membrane dividing the fibre into "compartments" corresponding to Bowman's discs. The same arrangement in "compartments" is also seen in the so-called "Festonbildung", where the fibre assumes the appearance of a string of pearls and the retracted parts correspond to the Z-membranes. Häggquist states that, like the sarcolemma, the basal membrane gives a connective-tissue-staining reaction and, as mentioned above, believes that these membranes fasten the fibre to the surrounding endomysium. Against this view, however, v. Möllendorff (1925) has objected that the staining method employed by Häggquist is non-specific; and other workers (Hürthle (1909)) have maintained on the basis of observations on living fibres that a basal membrane does not exist. We shall deal with this question again later, in connection with our own experiments. Otherwise it is not intended here to deal with the numerous histological data referred to in the literature, since the great majority of them were performed on fixed

and stained preparations. The method of fixation with glycerine-chloral hydrate employed and recommended by v. STUDNITZ has given no better results in our hands. A comparison of measurements using this method (Table II) with those stated in the later tables, and with similar comparative measurements made by LANGER (1937) show that the method is unsatisfactory.

Table II.
Experiments with Glycerine-Chloralhydrate Fixation according to v. STUDNITZ.

Preparation	A μ	I μ	A + I μ	
I	1.28	0.73	2.01	Without Fixing.
Other parts I	1.02	0.98	2.00	
II	1.69	0.53	2.22	Isometric Suspension.
Same Bundle, Other Fibres II	1.11	0.67	1.78	
Other Bundle II	1.19	0.51	1.70	
Same Bundle, Other Fibres II	0.72	0.34	1.06	
III	1.75	1.26	3.01	Small bundles isometric.

No further proof need be given that at present only those structures found in fibres which are living will be of interest when the functions of the muscle fibre are under consideration. As early as 1873 this was pointed out by ENGELMANN, and has been strongly emphasized since by HÜRTHLE (1909); but a perusal of the literature shows that only a very few workers have been guided by this obvious criterion. HOLTZ (1932) has indeed paid attention to this; but his results are only of limited interest, because his experiments were not performed under mechanical conditions that can be defined and reproduced.

When it is desired to investigate the microscopic structure

of the fibre under different conditions there are two further points to which attention must be paid. If it is necessary to record photographically changes taking place during contraction, the exposures must be less than $^1/_{10}$ sec. Such an exposure requires a very intense source of light of sudden action, especially where high magnifications are necessary as in the case of fibres with a small "height of compartment". These difficulties have been overcome by means of the "flash-light" lamp (Fig. 2) (Buchthal and Knappeis, 1935). For details the reader is referred to the original paper; it may be mentioned here that a considerable quantity of flash-light powder is ignited in a closed metal case provided with a silencer and outlet, so that the smoke formed during the combustion can be removed by means of a vacuum cleaner in a few seconds. The light is focussed on the mirror of the microscope by passing through two lenses and sometimes also a colour filter. The flash-light powder is ignited by a spark and the time of ignition is practically constant. By means of this lamp the time of exposure can be reduced to less than $^1/_{30}$ sec. and the amount of light emitted has proved to be adequate even where very high magnifications are employed.

A further requirement, and one which, in contrast to the above, does not depend on special technical aids, is that the mechanical state of the fibre in the moment of exposure shall be defined with certainty, and accurately reproducible. Disregard of this point is doubtless the cause of discrepancies so common in the literature, and of endless controversies. As with all other elastic bodies there will be some length at which the tension exerted by the muscle fibre is zero. In practice the procedure we adopted was as follows: The isolated fibre was subjected to the minimal

tension necessary to straighten it, i. e. to remove all kinks. The experiments shown below on passive extension of different degrees, followed by release of the fibre, show that even with varied material there is a close correlation between compartment height and resting length ("equilibrium length") so that the starting point is reproducible within

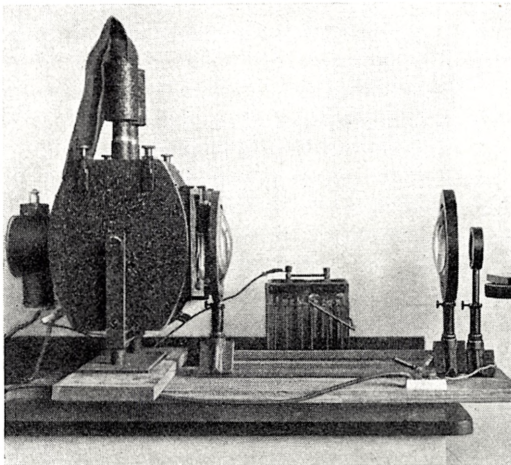

Fig. 2. Flash-light lamp.
(BUCHTHAL-KNAPPEIS).

narrow limits. If we consider the above position as the resting length, which in this case is the same as the equilibrium length, and determine all other starting lengths in relation to it, and if, moreover we make use of isometric contraction, the measurements will be properly controlled and experiments therefore reproducible. As will appear from Table III, isotonic contraction is, on the other hand, useless for this purpose.

The results will always show whether or not the contraction has been completely isometric; for, if so, the aggregate height of 10 or 20 muscular compartments, which

Table III.

Preparation	Rest			Preparation	Contraction			Isotonic Shortening
	A	I	A+I		A	I	A+I	
	μ	μ	μ		μ	μ	μ	in %
IX. B 351	1.32	0.85	2.17	IX. B 350	1.08	0.96	2.04	6.0
VIII. B 326	1.73	1.02	2.75	VIII. B 325	1.37	1.06	2.43	11.5
VIII. B 324	1.73	1.03	2.76	VIII. B 323	1.35	1.02	2.37	14.1
128	1.73	0.78	2.51	127	1.01	0.90	1.91	23.9

is of sufficient size to be measured from the photographs with considerable accuracy, should be identical in both the resting and the contracting fibre. At the conclusion of all experiments a control determination was made again with the fibre at its resting length (Table IV) to make certain that we had been working with living uninjured fibres.

Table IV.

Preparation	A	I	A+I	Extension	State of Fibre
	μ	μ	μ	in %	
VI. A 298	1.36	0.89	2.25	..	Length of Equilibrium.
VI. A 299	1.58	0.89	2.47	9.8	1. Extension.
VI. A 300	1.99	0.89	2.88	28.0	2. Extension.
VI. A 301	1.34	0.83	2.17	..	Length of Equilibrium.

Where it is desired to compare different conditions in the same fibre (passively extended, contracted etc.), care must be taken that in all circumstances it is the same part of the fibre that is photographed and measured. It is true that within the individual fibre, variations in the compartment height are very slight, but since the measurement of very small quantities is involved, possible sources of error, however small, must be excluded. Moreover, in this connection it should be borne in mind that the different fibres of a bundle often have different equilibrium lengths, and that in passive extension of the bundle we cannot be certain that all fibres will undergo the same degree of stretch.

In view of the above results, Buchthal, Knappeis and Lindhard (1936) have examined more closely the behaviour of the cross-striation in the frog's muscle fibre under varying experimental conditions.

An isolated fibre or a bundle of the frog's semitendinosus muscle was placed on a cover-glass in a drop of Ringer's solution and held in place by two fine glass or metal clips. The cover-glass was then inverted over a moist chamber. The fibre was also held in place by micro-electrodes, which were led into the chamber from below and the preparation could be observed through the cover-glass under the high power. The micro-electrodes, which consisted of Hg-calomel-potassium chloride electrodes connected with micropipettes filled with Ringer-agar, were adjusted by means of the micro-manipulator devised by Buchthal and Persson (1936). The micro-electrodes were used for extension of the preparation and for electrical stimulation.

The fibres were usually stretched so that a bundle was split in the shape of a V, one branch being fixed at equilibrium length, the other being stretched from 10 to 50 per cent of this.

An induction coil was used for stimulation, which was either limited to a single fibre (direct stimulation) or included the whole of the bundle (indirect stimulation). Where single contractions had to be recorded microphotographically the rapid sequence of stimulation and ignition of the flash lamp was secured by means of a Helmholtz pendulum. It soon appeared, however, that there was no demonstrable difference in the structure of the fibre during a single contraction and during a tetanus of short duration, and therefore in most cases it was preferable, for technical reasons, to employ tetanic stimuli of a few seconds duration. A

muscle fibre can stand repeated tetanic stimuli of short duration without alteration of its structure, but powerful stimuli of longer duration will produce a disturbance of the striation of the fibre. It appears to us unphysiological to apply to single fibres, as v. Studnitz (1936) has done, tetanic stimuli of some minutes duration. Our microphotographs have been taken either during a single contraction, or at the beginning of a tetanus of a very few seconds duration only. Intense stimuli will also produce disturbances for another reason than the above; namely that where larger bundles or whole muscles are employed there is a serious risk of displacement of those fibres situated in the field of vision, rendering a reliable estimation of the result of the experiment impossible. The effect of focussing on the measurements was carefully ascertained. It proved to be possible to vary the focus only within very narrow limits before the picture became blurred; and moreover that when sharply defined pictures were measured focussing was without demonstrable influence on the results.

For microphotography various cameras were used, especially Phoku-Kolibri (ocular 10 ×) with objective Zeiss Apochrom. 40 (N. A. 0.95) or 90 hom. Im. (N. A. 1.3). The photographic arrangement was calibrated photographically by means of a Zeiss object micrometer. A considerable number of measurements were made with different photographic materials in order to find plates with a sufficiently fine grain and to devise a suitable developing technique (Buchthal & Knappeis 1936). Negatives intended for comparable pictures were always treated in the same manner.

The measuring of the microphotographs was performed on positives from unenlarged negatives by means of a

Leitz ocular screw micrometer with movable line using 12 × magnification. One division of the micrometer then corresponded to 3.79 μ when parallax errors were eliminated. The measurements were made by two independent examiners on photographs, which, in order to minimise personal errors, were provided with random serial numbers. As a further check, photoelectric measuring was performed in several cases by means of a micro-densitometer.

The height of the isotropic (I) and the anisotropic layers (A), was measured, as well as the height of a single compartment and the total height of 10 compartments. The photographs were also examined for the presence of a basalmembrane (Z), the possible occurrence of Hensen's line in A, and any longitudinal fibrils or nod-shaped formations. Finally the thickness of single fibres, where visible, was measured.

The results of this series are briefly stated in the following tables.

All values given represent the mean of 10 determinations.

Table V shows comparative measurements of fibres at rest at their equilibrium lengths and during contraction. As will be seen, the variation in the single determinations in each column is very slight although it includes experimental errors as well as physiological variation in the material. The errors of the mean figures appear to be insignificant.

In the resting fibre the anisotropic disc is higher than the isotropic, namely 1.37 as compared with 0.81 μ; on isometric contraction A is shortened whilst I is lengthened. The aggregate height of a compartment will, of course, remain unaltered. A is thus 63 per cent. of the height of the

Table V.

Preparation	Rest A	Rest I	Rest A + I
	μ	μ	μ
I. B 263	1.35	0.74	2.09
II. A 267 a	1.33	0.83	2.16
II. A 267 b	1.37	0.91	2.28
III. A 271	1.35	0.89	2.24
III. B 275	1.33	0.79	2.12
VIII. F 335	1.41	0.76	2.17
IX. B 349	1.36	0.80	2.16
IX. B 353	1.48	0.79	2.27
Mean of 80 determinations	1.37 ± 0.008	0.81 ± 0.01	2.18 ± 0.01
Average error of a single determination	0.07	0.09	0.12

Preparation	Contraction A	Contraction I	Contraction A + I
	μ	μ	μ
I. B 262	1.11	0.96	2.07
II. A 266 a	1.12	1.02	2.14
II. A 266 b	1.14	1.09	2.23
III. A 270	1.07	1.17	2.24
III. B 274	1.06	1.02	2.08
VIII. F 334	1.23	1.00	2.23
IX. B 348	1.10	1.05	2.15
IX. B 352	1.21	1.06	2.27
Mean of 80 determinations	1.13 ± 0.009	1.05 ± 0.01	2.18 ± 0.01
Average error of a single determination	0.08	0.11	0.11

compartment in the resting fibre, I is 37 per cent. — In the case of the isolated contracted fibre the corresponding figures are 52 and 48, from which it would appear that during the contraction A is shortened by 18 per cent. whilst I is lengthened by 28 per cent.

Corresponding measurements on lizard's muscle at rest give as mean figures, A = 1.34 μ, I = 0.90 and thus the height of the compartment: 2.24 μ.

Passive extension of the fibre gives the results seen in Table VI.

Table VI.

Preparation	Rest A	Rest I	Rest A + I	Extension %
	μ	μ	μ	
IX. D 361	1.65	0.94	2.59	about 17.
VIII. G 339	1.69	0.87	2.56	
VIII. H 343	1.70	0.93	2.63	
VIII. E 333	2.00	0.99	2.99	about 40.
VIII. G 341	2.15	0.96	3.11	
VIII. A 322β	2.10	1.05	3.15	

	A	I	A + I
	μ	μ	μ
Unextended fibres	1.37	0.81	2.18
Extended fibres: Group I, Mean	1.68	0.91	2.59
Group II, Mean	2.08	1.00	3.08

	Proportional height A %	Proportional height I %	Extension A %	Extension I %	Extension A + I %
Unextended fibres	63.0	37.0	..	..	..
Extended fibres: Group I, Mean	65.0	35.0	22.6	12.4	18.8
Group II, Mean	67.5	32.5	51.8	23.5	41.3

Preparation	Contraction A	Contraction I	Contraction A + I	Extension %
	μ	μ	μ	
IX. D 360	1.38	1.15	2.53	about 17.
VIII. G 338	1.49	1.06	2.55	
VIII. H 342	1.39	1.17	2.56	
VIII. E 332	1.68	1.29	2.97	about 40.
VIII. G 340	1.70	1.37	3.07	
VIII. A 321β	1.46	1.63	3.09	

	A	I	A + I
	μ	μ	μ
Unextended fibres	1.13	1.05	2.18
Extended fibres: Group I, Mean	1.41	1.13	2.55
Group II, Mean	1.61	1.43	3.04

Table VI (continued).

	Proportional height		Shortening of	Lengthening of
	A %	I %	A in %	I in %
Unextended fibres	52.0	48.0	18.0	28.0
Extended fibres: Group I, Mean	55.3	44.7	16.1	24.2
Group II, Mean	53.3	46.7	22.1	42.0

As will be seen, A is more markedly influenced by the extension than I, 51.8 as against 23.5 per cent with 40 per cent. extension. If the passively extended fibre is made to contract isometrically, then A will be shortened and I lengthened; that is with 40 per cent. extension the changes are 22.1 and 42.0 per cent. respectively. The significance of these facts will be discussed later.

With regard to the degree of extension our experiments show that a passive extension of about 30, at most about 40 per cent. of the equilibrium length is reversible and without any permanent influence on the structure and function of the fibre. If the degree of extension is increased beyond 40 per cent. a disturbance of the structure occurs; first a displacement longitudinally of the contractile elements, relative to each other, their striations becoming indistinct, and then a complete disturbance of the picture, the fibre assuming the appearance of a sarcolemma filled with a granulated mass. Even before the latter stage has been reached, the fibre has lost its irritability (Fig. 3).

There has been general disagreement as to whether the A or the I-stripe is the broadest. However, the results of the above work on living fibres appear to show conclusively that under well-defined and reproduceable experimental conditions, the A-substance, both at rest and under passive extension within physiologic limits and

under isometric contraction, forms the greater part of the height of the compartment (Fig. 4).

The remaining problems over which there has been dispute are not so readily resolved. The question of the existence of the so-called basal membrane (the "Z-stripe") is of special interest, since the views expressed concerning this question are so divergent, some workers denying the existence of a basal membrane, others maintaining that it forms a continuous spiral throughout the whole length of the fibre and having the character of a nerve ramification (Tiegs (1922)). The latter views will not be discussed in detail here; but as to a possible helicoidal arrangement it may be mentioned that this idea is recently supported by Aurell & Wohlfart (1936) from Häggquist's laboratory. This work which was performed on fixed and stained material, appears open to criticism and is, moreover, so decidedly at variance with functional viewpoints, especially Buchthal & Péterfi's potential measurements (see later), that we cannot accept the authors' conclusions. On the basis of his experiments with hydrophilus muscles Hürthle regards the Z-membrane as a structure that is absent from the living fibre but only appears when the fibre is moribund. We have been unable to confirm this statement in observations on frog's muscle where stimulation before and after photographing proved that the tissue was in normal condition. It is true that the Z-stripe is not invariably seen in the living fibre, but it is frequently seen (Fig. 5) and under conditions which certainly do not suggest that the fibre is abnormal.

The fact that photographs of the stripe are only successful in a minority of cases is due to the fact that the Z-stripe

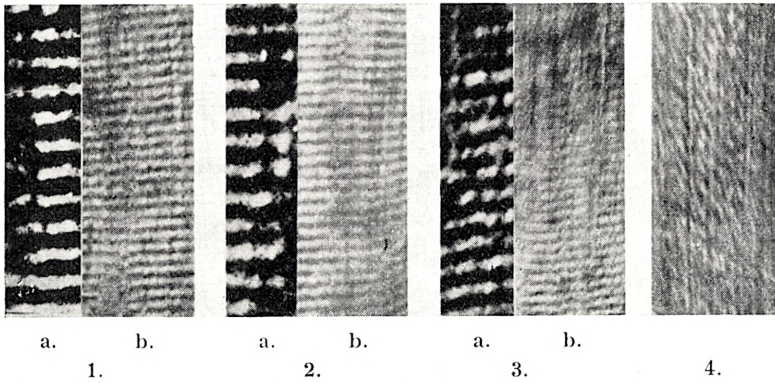

Fig. 3. Structure of resting living fibre with the following degrees of extension:

1. stretch over 23 % of resting length.
2. — — 34 - - — —
3. — — 39 - - — —
4. — — 50 - - — —

a. = magnification × 1000; b. = magnification × 600.
(Buchthal, Knappeis & Lindhard).

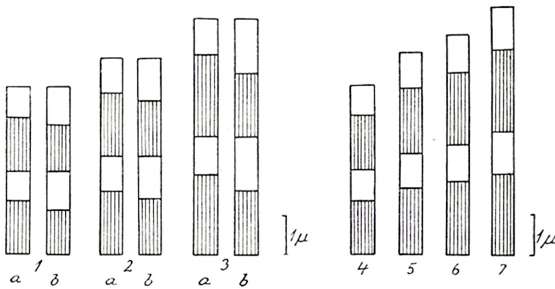

Fig. 4. Relation between A and I substance in the resting, stretched and contracted fibre (diagrammatic).

a. = at rest; b. = isometric contraction.
1. fibre at resting length.
2. — 25 % stretched.
3. — 40 - —
4—7. resting fibres stretched from 0—45 % of resting length.
(Buchthal, Knappeis & Lindhard).

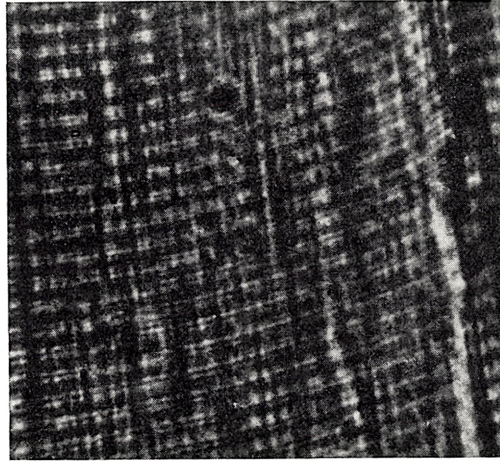

Fig. 5. Microphotograph of living single fibre with visible Z-membrane. Magnification 1000 ×.

only appears distinctly as the focus is changed, which cannot be done in the course of photographing. According to statements by v. MURALT after WOHLFART (1933), who examines the fibre by means of ultraviolet light and a quartz optics system, the Z-stripe is always found in the living fibre. CIACCIO (cit. PÉTERFI, 1937), has found that during metamorphosis in frog larvae, the basal membranes in the otherwise empty sarcolemma persisted after the myofibrils had been resorbed. It may be added that BUCHTHAL and LINDHARD in unfinished experiments on the micro-incineration of single fibres, have in several cases been able to demonstrate a distinct Z-stripe in the reproduction of the striation seen in the "ash-picture"

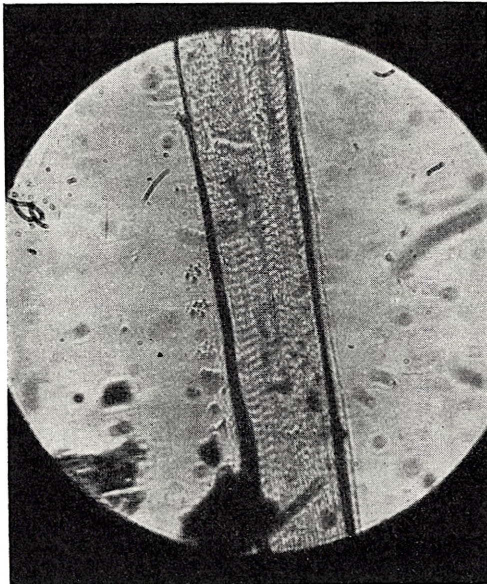

Fig. 6. Ash-picture of single muscle fibre, obtained by micro-incineration.

(Fig. 6). In the latter case we are not concerned with living fibres, nor even with fibres at all; yet this observation implies that in the part of the fibre where the Z-stripe is seen there is something of a substantial nature differing from the surroundings. The same fact appears from Häggquist's staining experiments, which show that the Z-stripe is stained like the sarcolemma; and even though the staining method employed was not a specific one this does not affect the point at issue. Lastly Bowman has shown that fibres treated with a KOH-solution of suitable strength fall apart into transverse discs limited by the basal membranes.

To these histological grounds for the existence of the Z-stripe as a pre-formed, normal constituent of the fibre there must be added the important evidence from physiological observations. Electrostatic measurements of single fibres by Buchthal and Péterfi (1934) have shown that the potential difference between the two electrodes increases in proportion as the number of intervening muscular compartments; on extension of the fibre the potential difference decreases, and disappears completely when the Z-stripe is disturbed. This fact implies some kind of an isolation of the compartments from each other, which is difficult to imagine except with the aid of some structural elements. As already mentioned, it must be supposed on physiological grounds, that an intimate connection exists between the sarcolemma and the endomysium, and it seems logical in agreement with Häggquist to refer this connection to the basal membrane. Finally, the basal membrane probably serves as a point of fixation for the muscular compartments during contraction. In this connection it is not sufficient to suppose that by means of these membranes the fibre as a

whole is attached to the endomysium, leaving the fibrils free. When it is remembered that in a conical fibre during contraction, one end is extended and the other shortened, it becomes difficult to see how a fibril 20 mm. long, containing about 10^4 alternately isotropic and anisotropic discs, would be able after contraction to rearrange itself in relation to the adjacent fibrils, so that the striation remains distinct. After the contraction the striation is as distinct as before, unless the fibre has been injured by strong stimuli applied for a longer period. Therefore, the suggestion that the basal membrane may be fenestrated in order to allow the passage of the fibrils seems improbable. And such fenestration has actually never been observed. On the other hand both Péterfi (1937) and Nagel (1935) describe the sarcolemma, which we may consider to be of structure similar to the basal membrane, as consisting of a network of filaments, the meshes of which are filled with homogeneous substance. Such an observation could be interpreted as fenestration. However, the fibrils though no doubt continuous, are "linked" by the basal membranes.

We do not know the details of the character of the basal membranes, but Häggquist's supposition that they consist of collagenous substance has some foundation in fact, inasmuch as the tissues separating the single plates of the electric organs of fishes are known to be of a connective tissue character. Likewise it seems probable that the thickened sarcolemma covering the motor end-plates (the telolemma) serves as an electrical insulator between end-plates and adjacent fibres. But this does not imply that the inter- and intra-fibrillar basal membranes are of the same thickness or in the same physico-chemical state. When the fibre is treated by Bowman's method it falls apart in transverse

discs, the basal membrane being dissolved as a whole; but when the fibre is boiled, at any rate if the boiling is not carried too far, the fibre is disorganized into fibrils, the intra-fibrillar membrane remaining intact, when the sarcolemma is torn. Corresponding with APATHY'S (1907) & PÉTERFI'S view this fact shows that the inter- and intra-fibrillary parts of the Z-membrane must be of different natures. Again, in decomposing fibres it may sometimes be observed that some fibrils (sarcostyles) retain the power of contraction whilst others have already lost their irritability. In this way longitudinal displacement of the different fibrils will take place, the striation being so considerably displaced that a rupture of the basal membrane between the active and the inactive parts of the fibre must be inevitable.

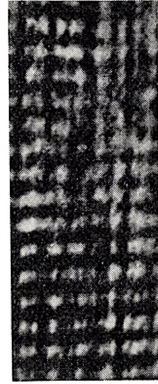

Fig. 7. Longitudinal striation in a single fibre. Magnification 1000 ×. (BUCHTHAL, KNAPPEIS & LINDHARD).

Although in such cases as these there can be no doubt as to the existence of longitudinal fibrils (sarcostyles), the longitudinal fibrillary striation in the living fibre is generally not nearly so distinct as the cross striation. It is, however, possible by means of ordinary illumination to ascertain the presence of longitudinal fibrils which are about 1.5 μ thick. We do not find a completely well-defined transition between the A- and the I-stripe in the single fibril; which agrees with W. J. SCHMIDT'S (1937) and D'ANCONA'S (1931) views on the distribution of the anisotropic substance; whilst the longitudinal striation can be followed from one basal membrane to the other (Fig. 7).

On the basis of our investigations in connection with the mechanical phenomena we must suppose that the

individual muscular compartments consist of rod-shaped formations (fibrillary sections) with a greater or smaller amount of intervening sarcoplasm. In the middle the rod-shaped formations consist of A-substance, at either end of I-substance. The rods forming the highly anisotropic A-substance are on an average 1.6—1.7 μ long and 1.3 μ thick, and are probably composed of a number of contractile elements. As previously mentioned (p. 25), A is shortened during isometric contraction, and I extended; as this takes place simultaneously in all compartments under normal conditions, they will act as a single unit, the tension exerted being transmitted to the tendons via the basal membrane and the endomysium.

A question of considerable interest is that relating to the volume of the fibre during contraction. Here, also, there exists considerable uncertainty. Most workers have either found the volume to be constant, or very slightly changed. Buchthal, Knappeis and Lindhard, too, found the volume of the muscle compartment to be constant during contraction, the thickness of the fibre being unchanged; but these experiments were not primarily concerned with the determination of possible changes of volume. Some of the most recent and best observations in this field, those by Meyerhof and Möhle (1933) and Meyerhof and Hartmann (1934) give as their results that during contraction, when the muscle is covered by liquid paraffin during the experiment, there is a slight constriction of volume, which in the authors' opinion might be explained by the chemical changes taking place during the period of restitution. In the case of isometric contraction the volume changes amount to 2.0×10^{-5}, in the case of isotonic contraction to 6.4×10^{-6} cm.^{3} per gram of muscle. In other words the change of volume

is 3 times as large in isometric as in isotonic contraction, a difference that is difficult to understand if the diminution in volume is considered to be a direct result of the chemical changes, unless the tension in the two cases presents corresponding differences. Volume changes of this order could, however, hardly be detected with the technique employed by BUCHTHAL, KNAPPEIS and LINDHARD. Other workers, however, (E. FISCHER (1935)) have claimed to be able to demonstrate a slight increase in volume; moreover, such an increase, even of a very considerable size, is shown indirectly in the measurements made by HÜRTHLE.

If calculation is made from HÜRTHLE's mean figures the increase in volume of the fibre during contraction amounts to no less than 38 per cent.; and if his single measurements be used even 50 per cent. increases may be indicated. An increase in volume of such an order has no real existence; such increases in size if they occurred would be easily detected in our experiments; but in all cases where we have been able to measure exactly the thickness of completely isolated fibres we have, in the case of isometric contraction, found the volume of the muscular compartment to be unaltered. In such cases the volume of A or I is thus proportional to the height of the respective sections. The discrepancy between our own and HÜRTHLE's results are, we feel, due to the unreliable nature of HÜRTHLE's measurements of thickness, either because the fibres had not been isolated, or because they were not normal. It is not permissible to measure, as he has done, "resting values" in one section of a partially contracted fibre, as the dimensions of the passive parts of the fibre may be influenced by the contracting parts. Moreover the possibility cannot be completely excluded that the cover-glass may have compressed the con-

tracting parts of the fibre, making the latter appear thicker than it really is. Finally, when in the muscle fibres of insects which normally react very rapidly, slow localised contractions are found to occur, such a reaction must be considered abnormal in character; in such circumstances it cannot be assumed that in other respects the fibre will react normally or display normal conditions of diffusion.

Minute Structure.

The study of minute structure is concerned with the constituents of the smallest tissue elements visible under the microscope. These are the structural elements intermediate in size between these and the molecule, the molecular aggregates once termed "micellae" by Nägeli. The methods that have come into use for observation in present fields of research are determinations of double refraction, roentgen-optical methods, and thermoelastic methods. It seems to us, however, that the latter methods have not been employed with the necessary reservations. As to the roentgen-optical methods it seems preferable, at the present stage of development of the technique, to confine its uses to the examination of myosin. The muscle "mummies" that are so often examined are of little use in the study of the living musculature, and this is especially so when muscles of quite different types are used indiscriminately. As to thermo-elastic experiments, it should be borne in mind that the results of heating living tissues may be entirely different from those obtained on dead material. The living tissues, which at present must be studied through their metabolism, react to changes of temperature with far more complex reactions than do the physical objects usually employed and, therefore,

erroneous conclusions may easily be drawn, especially so long as our knowledge of them is imperfect.

It has long been known that the A-substance of the muscle fibre is anisotropic, and hitherto the belief has been wide spread that the I-substance is isotropic; more recent investigations by W. J. Schmidt and d'Ancona, however, seem to indicate, as already referred to, that the I-substance, also is anisotropic, though to a far less degree than the A-substance. Whilst a rod-like structure can be demonstrated with some certainty in the A-substance we have no certain knowledge of the structure of the I-substance. According to measurements performed by Buchthal, Knappeis & Lindhard A is shortened during isometric contraction and I is lengthened, but this does not necessarily mean that A alone is contractile, as a faint contraction of I might be masked by contraction of the more bulky A-layer. Any contraction of I, however, must be of minor importance. Many years ago Engelmann (1873) maintained that contractility, not only of the skeletal muscle but also of other contractile substances, was always associated with the occurrence of anisotropic uniaxial bodies arranged parallel to the direction of contraction of the elastic substance. Such bodies can be demonstrated in A, but it must not be concluded that they are not therefore found in the I-layer at all.

H. H. Weber (1934) and Noll & Weber (1934) have performed comprehensive investigations comparing the double refraction of very fine myosin filaments with the A-substance of the muscle fibre, and have found a very far-reaching correspondence between the minute structure of the two. This applies especially to the so-called "Rod-double refraction" ("Stäbchendoppelbrechung") (Wiener), which must be attributable to the difference in refractive

index between the rods and the surrounding medium. As shown in Fig. 8, this "Rod-Double-Refraction" diminishes as the refractive index of the medium approaches more and more to that of the Rods. However, the position of the lowest point on the curves shows that some Double Refraction always remains, which is due to an inherent property of the molecular structure of the rods. Values of the latter component obtained for myosin threads may not be comparable with those for intact muscles, since the conditions affecting the protein molecule may be quite different in each case.

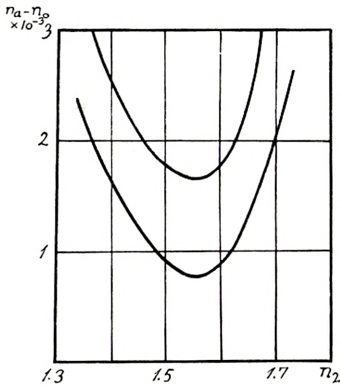

Fig. 8. Striated muscle (lower curve) and myosin thread (upper curve). Ordinate: total double refraction measured after immersion in fluids of different refractive indices. Abscissa: refractive indices of the fluids. (E. Fischer and H. H. Weber).

According to Weber a myosin thread, which contains 20 per cent. of protein, is composed of longitudinal micellae, containing 70—80 per cent. of protein and separated from each other by waterfilled interstices. The distance between the individual micellae, is only a few $\mu\mu$, so it cannot be supposed that there would be room for other protein molecules in the interstices. Weber estimates the distances between the filamentous molecule chains composing the micellae to be about 11 Å. Each micelle is supposed to contain 10—20 "main-valency-chains" ("Hauptvalenzketten"), the dimensions of each being: thickness 4 $\mu\mu$, length 50—100 $\mu\mu$, and volume about 1100 $\mu\mu^3$. The molecular weight of myosin is said to be of the order of 10^6.

Though Weber gives many good reasons for supposing

that the A-sections of the fibrils of the cross-striated skeletal muscles consist of myosin, this does not necessarily apply to the A-sections of the muscle-fibre, for the fibrils must be supposed to be embedded in a greater or smaller amount of sarcoplasm. WEBER's statement of the quantitative proportions in the muscle is inaccurate, since his contentions are based on incorrect statements of the proportion between the dimensions of the A- and the I-segments. As to the amounts of the different proteins he considers that about 40 per cent. of the protein is myosin; to this must be added 15 per cent. of myogen, 15 per cent. of globulin, and nearly 30 per cent. of stroma-protein. WEBER does not say how these substances are distributed between the I-substance of the fibrils, the sarcoplasm and the stroma; his supposition that the I-section of the fibrils consists of stroma-proteins is probably also derived from the erroneous statements concerning the relation between the A- and the I-substance during contraction, found in the literature.

v. MURALT (1932) has recently examined the double refraction of whole muscles in isometric contraction and has tried to establish the time relation between bi-refringence and the mechanical response by means of a method giving a reliable record of the changes in double refraction. Among other things the experiments showed that the variations of double refraction preceded the development of tension.

WEBER's work has usually been performed on fixed material, which it has been emphasized several times, has numerous drawbacks. Other experiments, chiefly owing to the use of whole muscles, have not given quantitative results. Therefore BUCHTHAL & KNAPPEIS (1937) have re-determined double refraction on living material under various conditions. The bi-refringence of resting, passively extended and con-

tracting fibres was examined by means of a Babinet-ocular compensator.

The double refraction can be calculated according to the formula:

$$n_a - n_o = \frac{\gamma\lambda}{d},$$

where γ is the phase difference, λ the wave length of the light employed, in this case 546 $\mu\mu$, d the thickness of the object determined by means of an ocular screw micrometer, and n_a and n_o the refractive indices of the ordinary and the extraordinary ray respectively. Fig. 9 shows the course of the stripe of interference when the light passes through a muscle fibre, and the displacement of this line is a measure of the bi-refringence. In the case of a single living fibre of the frog's semitendinosus it is:

$$(1.70 \pm 0.016) \cdot 10^{-3}$$

as a mean figure from 230 preparations examined. As the thickness of the fibre and the difference in phase change proportionally, the quotient $\frac{\gamma\lambda}{d}$ remains a constant. Injured fibres give far greater values of bi-refringence (up to $2.30 \cdot 10^{-3}$), which taken in conjunction with the structural changes makes such preparations easily recognizable. With advancing, decomposition of the structure the double refraction, however, also decreases considerably; it may then become uniform throughout the whole of the fibril, which

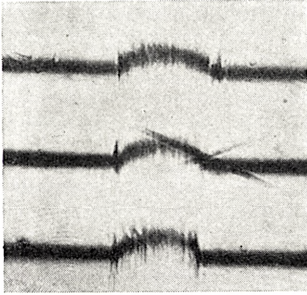

Fig. 9. Bi-refrigence of a single living muscle fibre measured with Babinet-Compensator. (Buchthal & Knappeis).

apparently has caused some workers (Meyer & Hürthle) to believe that the whole of the fibril should consist throughout its length of more or less orientated myosin micellae. Similar phenomena may appear when whole muscles are used for the experiments instead of isolated fibres. Therefore, neither whole muscles nor injured fibres can be employed for determinations of double refraction. Experiments with vital staining (Pischinger) have shown that the A- and the I-segments stain differently and must thus consist of different substances. Moreover H. H. Weber has put forward sound reasons why the myosin is found exclusively in the A-segments. The structure of the I-segment is unknown, but this substance too may on mechanical grounds be expected to be composed of molecules longitudinally orientated or arranged in reticular formation. Buchthal & Knappeis found no difference between the double refraction of the resting and the passively extended fibre, which is apparently due to the fact that the decrease in the double refraction which may have occurred during the extension, has been compensated by the decrease of the thickness of the fibre which results from stretching. During, and after the contraction, fluctuations in the double refraction occur, which in their time relations closely resemble the fluctuations occurring in fibre potential and electrical resistance. A corresponding analogy between the double refraction and the fibre potential is found in the case of the effects produced by different substances applied to the fibre. For example, lactic acid in a suitable buffer mixture gives rise to a simultaneous, reversible decrease of the fibre potential and of the double refraction. This observation suggests that there is a close relation between potential difference and bi-refringence which in turn is intimately concerned with the contractile substance (Fig.

10). Buchthal & Knappeis have also made use of the changes in double refraction which take place when the muscle fibre is influenced by different substances, to obtain direct information as to the permeability of the fibre itself to substances in the external fluid. Bi-refringence is thus employed as an actual indicator of the penetration of sub-

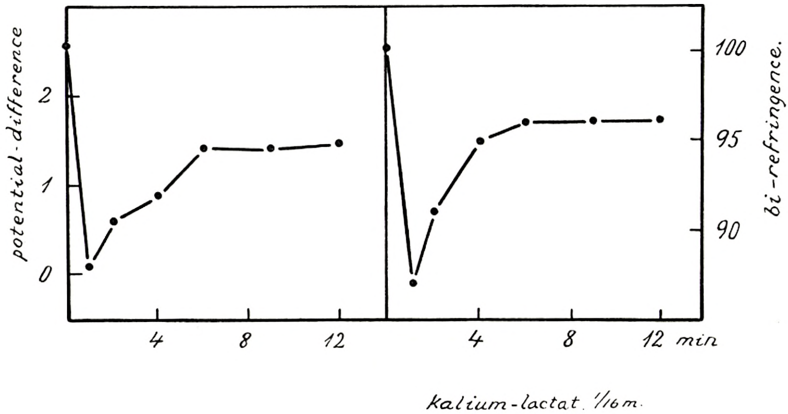

Fig. 10. Bi-refrigence and potential difference of a single fibre after application of potassium lactate. (Buchthal).

stances into the fibres. It was found that acids cause a diminution of the bi-refringence, which progresses constantly with increasing concentration and time of exposure (Fig. 11). On the other hand it seems that the possibility of a specific anion effect on the double refraction can be excluded. Anions and cations invade the fibre in equivalent amounts. Changes of the double refraction under the influence of neutral salts must be supposed to take place by exchange of anions, e. g., the carbonate ion is exchanged with lactate ion in the external fluid, causing a change in the pH of the contents of the fibre. But it is improbable that an exchange of cations takes place, since phosphoric acid, even in high concen-

trations, does not influence the double refraction, whilst lactate—and acetate—RINGER's fluid with a pH of 7.4 causes a spontaneous, reversible decrease of the double refraction.

As to the roentgen-optical methods ASTBURY and his collaborators in a series of publications, have especially contributed towards the elucidation of the minute structure

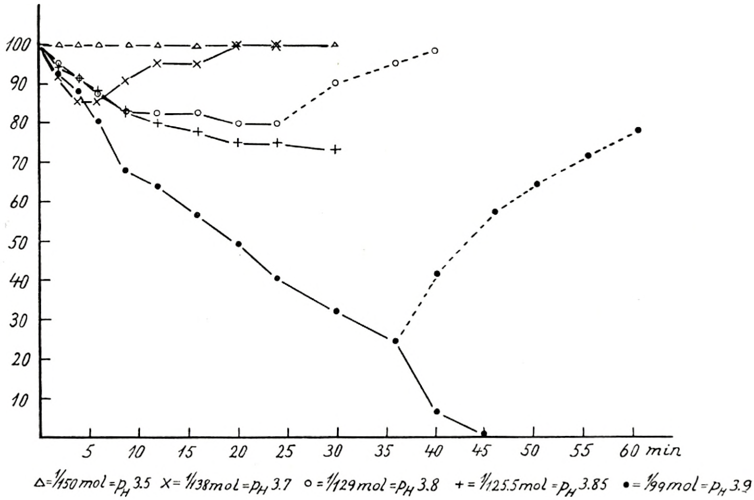

Fig. 11. The influence of lactid acid in different concentrations upon bi-refringence. Stippled lines: recovery after washing in Ringer. (BUCHTHAL & KNAPPEIS).

of the myosin. ASTBURY & DICKINSON (1935) have moreover demonstrated a certain analogy between the structure of myosin and keratin; but for the time being it must be left as an open question whether this demonstration is of any value to the understanding of the muscle function, for although much is known of structure of dead myosin threads, little can be said of the state of myosin in the living fibre.

KURT H. MEYER's investigations are in the main applicable to biological problems. MEYER & FERRI (1936) have examined the relation between the elastic properties of a

body and its molecular structure, especially with regard to elastic and collagenous connective tissue. Previously K. H. Meyer and his collaborators examined carefully the thermoelastic conditions in rubber and were able from their experiments to draw certain conclusions as to the force with which stretched rubber will contract. Wöhlisch (1932) and others from their work on different organic tissues, have found that the yellow cervical ligament of the ox will contract when heated within certain limits, whilst tendinous tissue lengthens. Meyer & Ferri have attempted theoretically to show how, on the basis of the thermoelastic conditions of a substance, we can analyse the force of contraction of the passively stretched material into its component forces. The authors draw attention to the fact that the amount of work any system is able to perform is determined by its internal energy, the attractive forces between the single elementary constituents, and the molecular movements, as is also the state of equilibrium of the system. What the system loses in internal energy it will gain in molecular movement, i. e., in heat; the movement of heat tends to produce complete irregularity in the arrangement of the molecules, and its proportion in any amount of work performed will, therefore, become greater the more organised the structure of the substance is. Any system in which the molecules display a certain orientation has a lower entropy than the corresponding system in which the molecules are arranged in less orderly fashion. With increasing temperature the internal energy will decrease whilst the heat movement will increase. The authors establish the equation

$$dA = dE - T\,dS,$$

in which A is the free energy of the system, E the internal energy and S the entropy, and determine dE and $T\,dS$ by

means of the temperature coefficient of the force of contraction. Further consideration of these formulae need not be made here; the question of special interest in this connection, is that of the conclusions which can be drawn from the experimental results regarding the molecular structure of the substance. Thus dE denotes change in the internal potential energy between the molecules or parts of the molecules. If this quantity is negative during the contraction, it indicates that internal forces are absorbed e. g. through crystallization, whilst a positive value for dE is indicative of a process in the opposite direction, i. e. the transition from the crystalline to the amorphous state. If, lastly, dE is equal to 0 it must be supposed that the contraction is due exclusively to the movement of heat.

If S increases when the substance is passively stretched, it means that through the stretching the atoms will obtain greater freedom of motion, especially in the direction of the extension; if, on the other hand, S decreases on extension but is increased during the contraction, as is the case when rubber is stretched under certain conditions, it must be supposed that the freedom of motion of the atoms decreases on extension owing to an orientation in the direction of this extension. It is well known that vulcanised rubber consists of a mass of long flexible "main-valency chains" ("Hauptvalenzketten") united into a network by means of sulphur bridges. When the substance is passively extended these molecular chains will arrange themselves parallel to the direction of extension, gliding towards each other as in a viscous fluid, and the movement of heat will try to reestablish the previous chaotic state. The requirement for contraction is, however, that the substance shall consist of long "main-valency chains" in which the molecules, or

parts of molecules, are combined through very firm valencies. If these unions do not exist, the molecules may be disarranged solely through turning, no change of the outer shape being necessary, as is the case with dipoles orientated in an electric field when the action of the field ceases.

When these considerations are applied to the results of experiments with elastic connective tissue and tendinous tissue it appears that elastic connective tissue consists of long, flexible "main-valency chains" which, on passive extension, become orientated in the direction of the traction, whilst the movement of heat re-establishes the disorderly condition during the contraction. Tendinous tissue also consists of long "main-valency chains" but they are already orientated longitudinally when the tendon is at its length of equilibrium and so cannot be stretched further by passive extension. In a more recent publication, Meyer & Picken (1937), have tried to extend to muscles the results obtained by them in observations on various organic substances. Though realizing that the muscle consists of different heterogeneous constituents the authors believe that the thermoelastic methods of examination may still be employed with advantage, since by their aid information can be procured about precisely those substances which are responsible for the contraction. We consider, however, that this view is improbable. The force of contraction measured by Meyer & Picken is the resultant of several components which, at any rate quantitatively, are completely unknown. Moreover the authors work with muscles of vertebrates and invertebrates indiscriminately and draw conclusions from one kind of muscle tissue to the other, a view that has resulted in a great number of misconceptions. Finally, they take for granted that as regards minute structure the resting muscle

can be compared with unstretched rubber; this, see later, is not supported by our results. Despite objections which may be raised to the above work, it must be granted that the authors observations show that the muscle, or perhaps more correctly certain parts of the muscle fibre, must be composed of long, flexible main-valency chains, which become orientated longitudinally on passive extension and revert, at any rate, to a less extended condition when the muscle is allowed to resume its resting length.

As to the sarcoplasm no conclusions whatever can be drawn from our experiments. It is known that the sarcoplasm contains myoglobin and it must also be supposed to contain the glycogen of the muscle, as there would scarcely be room for the large glycogen molecules in or among the myosin micellae. Therefore a nutritive role may be suggested for the sarcoplasm. The suggestion that the sarcoplasm possesses contractile properties is so far removed from facts of histology or physiology that it need not be discussed here.

The Motor End-Plate.

Anatomically the motor end-plate belongs to the muscle fibre, being situated beneath the sarcolemma. Functionally it is so closely connected with the muscle function that it becomes impossible to examine and describe the latter except in conjunction with the function of the end-plate. From a phylogenetic point of view the end-plate must, however, be considered an organ sui generis, as a structure that both ontogenetically and histologically is homologous with the electric organs of fishes. A single summarizing statement by Babuchin (1870) will suffice: "...... dass die elektrischen Organe eigentlich Muskeln sind, aus denen nur die Muskelsubstanz entfernt ist; und umgekehrt, die Mu-

skeln sind elektrische Organe, in welchen unter allen Platten Muskelfasern eingeschoben sind . . . . . Die elektrischen Platten und die motorischen Endplatten sind in morphologischer Hinsicht identisch." This view has been well confirmed by later investigations of Ewart (1892) into the development of the electric organ in Raia, and also by Boeke's observations (1927) on the development of the motor endplate. In mammals, birds, reptiles and fishes the motor end-plate is an organ shaped like a biconvex lens with a diameter of 40—60 μ. Its size appears to have no relation to the size of the species of animal in question but seems proportional to the thickness of the muscle fibre. It is not bounded on the side towards the fibre substance by any membrane demonstrable by histological methods; it is covered by the sarcolemma, which is strengthened by the covering of Schwann's and Henle's sheaths of the nerve filament (the telolemma of Kühne). The end-plate consists of embryonic fibre substance in which there is a number of great, ellipsoid nuclei with 1 or 2 nucleoli (the "Sohlenplatte" of Kühne). The nuclei are formed by metamorphosis of the nuclei of the muscle fibre. To this "sole" comes a somatic motor nerve branch, the external sheaths of which, as already mentioned, pass into the sarcolemma, whilst the medullary sheath disappears and the axis cylinder alone perforates the sarcolemma, whereupon it breaks up into numerous fibrillary loops in the sole.

In several publications Boeke has maintained that the actual distribution of nerves does not take place in the end-plate but that beneath this, in the sarcoplasm, there is another network of extremely fine neuro-fibrils, which in turn are connected with the myofibrils. On this view then, the end-plate is not a terminal organ, and the whole of this

histologically well-known and well-characterized structure would therefore be without any biological meaning whatsoever. BOEKE's nervous network has no foundation, in fact, either. The specific substance of the neuron is in fact delimited from the effector cell as the classical neuron doctrine states (PÉTERFI, (1935)). PÉTERFI moreover points out that the potential difference between end-plate and muscle fibre demonstrated by BUCHTHAL & LINDHARD could hardly exist if there was a continuous transition from neuroplasm to cytoplasm. Furthermore, BOEKE's conception is incompatible with our knowledge of curare, and it is quite unphysiological to imagine a connection at random between ultramicroscopical nerve branches and any muscular compartments.

Mechanical and electrostatic findings make it necessary to assume that the muscle fibre consists of compartments, which are isolated from each other. As the height of these compartments is 2—2.5 μ there will thus be about 25,000 compartments in a muscle fibre about 5 cm. long, each compartment containing something like 12—1300 fibrils. It must either be supposed that the natural stimulus proceeding from the end-plate is propagated to the whole of the fibre without special conducting tracts, or it must be supposed that each of the units mentioned above is innervated separately, a view which to say the least appears improbable. The most serious objection to BOEKE's theory is, however, the fact that the secondary network of nerves in the fibre cannot be demonstrated with any degree of certainty (cf. J. V. WILKINSON (1934)). The only picture which may be relied upon is obtained by making transverse sections through end-plate and fibre; but the very few pictures of such a transverse section found in BOEKE's publications (as

far as we know only 2), do not display secondary nerve ramifications under the end-plate (Fig. 12). These networks are not seen, either, in exact longitudinal sections and the shadows appearing in obliquely projected longitudinal sections prove little. Such appearances may often be found when we are approaching the limits of resolving power of the microscope, and might very well be artefacts produced through fixing and staining. For the time being it may be supposed that the irregular shadows which at present form the basis of Boeke's so-called secondary network of nerves, belong to this category.

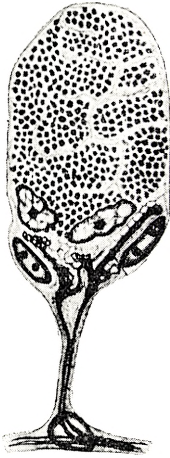

Fig. 12. Cross-section of end-plate and muscle fibre. (Petersen).

In amphibia the somatic motor nerve distribution is of a somewhat different form, the end-plate not appearing as a circumscribed lenticular body, but the axis cylinder being seen to end in a root-shaped ramification ("Stangengeweih" of Kühne), covering a relatively large area. The sole follows to some extent the nerve branches, to which also the nuclei connected with the end-plate attach themselves. The difference between this form of the terminal organ and the typical form described above is thus a purely morphological one.

As a rule there is one end-plate on each fibre, in most cases situated near the middle of the fibre or, in the case of conical fibres, a little nearer the thicker end. In certain forms of fishes the end-plate is, however, situated near the end. Some workers e. g. Agduhr (1916), describe 2, even 3 end-plates on each fibre, a phenomenon associated with plurisegmental innervation of the fibre. This question,

on the elucidation of which a considerable amount of work has been expended, does not, however, seem to be of corresponding functional importance and, therefore, the details will not be considered here.

Besides the somatic motor end-plates we find, usually in close proximity, a small end-plate of similar structure, to which a non-medullated nerve fibre leads; but the question of the autonomic innervation of the muscle fibre is not so far clarified histologically that we can deal with it in detail here.

Boeke makes the following statement about the development of the organ. Even before the individual muscle fibres are differentiated the motor nerve grows into the embryonal muscular plate. The nerve forms a network, the greater branches of which run across the muscle fibres whose cross striation has now become visible. Moreover the muscle nuclei have now begun to move from their original central position towards the surface of the fibres. The sarcolemma has not yet been formed. At this juncture a thickening develops on the transverse nerve branch, corresponding to the individual muscle fibre. The thickening develops into a fine fibrillary network, gradually moving away from the nerve branch and ultimately being connected with the latter only by a thin stalk. Simultaneous with this development the sole is formed from the embryonal protoplasm, and muscle nuclei invade it and are then transformed to nuclei characteristic of the motor end-plate.

Previously it was taken for granted that the essential part of the end-plate was the ramification of the axis cylinder, and the muscular part of the organ, the sole with its nuclei, was considered as something accessory, unimportant, or even as a resistance interposed between the nerve ramifica-

tion and the contractile fibrils (E. du Bois-Reymond). Ranvier compares the sole with the glass of a Leyden jar, a comparison perhaps more allied to functional viewpoints. However, a few workers (e. g. Babuchin) had noticed that the intensity of the discharge in the electric fishes did not appear to be in direct proportion to the extent of the nerve ramification in the electric plate, whilst on the other hand it increased with the mass of the sole. Thus Babuchin finds that the electric "shock" from a young malapterurus is much weaker than the shock from an older animal, though the young animal has the full number of electric plates and the nerve ramification is practically fully developed; the part of the organ that increases in mass with the growth of the animal is the sole. It appears highly probable that this view of the sole, as the structure characteristic of the motor end-plate, is the correct one. As mentioned previously, there is no demonstrable limiting membrane between the sole of the end-plate and the remaining fibre substance; but the electrostatic determinations to which we shall refer later, show that a limiting boundary exists there which permits the maintenance of a fairly considerable potential difference between the end-plate and the remaining part of the fibre.

The Elastic Properties of the Muscle Fibre.

The elasticity of muscle has been the subject of investigations that have gone on for more than a century, and still no general agreement has been reached in results. The determination of elastic moduli by physical methods on a complex living subject such as the muscle has been and is still the centre of much debate, and there is no general agreement as to the usefulness and suitability of the various methods usually employed.

It may be permissible to determine the modulus of elasticity of a muscle, a bundle or a fibre by the usual methods provided the object in question is considered as a homogeneous physical object. In a similar fashion the technologist determines the modulus of elasticity of oak etc. In either case it is impossible either in theory or in practice to take account of the different structural elements of which the object is composed. It has been maintained that the contractility of the muscle should interfere with determinations of elasticity; but this will not be so if care is taken that the experimental technique employed does not stimulate the muscle. Resting and active muscles can simply be considered as different physical objects whose elasticities are determined separately under precisely defined experimental conditions. The existing determinations of the elasticity modulus of muscle give widely fluctuating absolute values, which is not to be wondered at, because it must always be extremely difficult, if not impossible, to obtain a series of preparations which are in the same condition, if only for the reason that we possess no criterion of this and are therefore unable to control it. But we can obtain reliable relative elasticity moduli for one and the same muscle in the resting and active states. In all cases care must be taken that the changes in the muscle are reversible and that the experiments are reproducible. This as a rule presents no difficulties.

The criticisms directed against these methods have most often been evoked by cases of their wrongful use. In certain cases, however, objections may be raised on theoretical grounds, e. g. against the extension method, since the marked elastic after-effects of the muscle may easily be responsible for unreliable measurements. However, even by means of this method comparable results have been obtained. The

same applies to Bethe's elastometer. In its original form this apparatus was unsuitable, as tension was measured instead of elasticity; but Steinhausen has succeeded in modifying the apparatus so that it can be used for determination of elasticity. Objections can also be made against the apparatus employed by Gasser & Hill.

Apart from such errors as omitting to determine one or more of the factors concerned in the formulae used in calculations of the elasticity, the most common experimental error is overloading of the preparations, often to an absurd degree, and omitting to employ definable and reproducible experimental conditions. These errors are to some extent due to the fact that often preparations quite unsuitable for the purpose have been employed, as for instance whole gastrocnemii, the longest fibres of which are about 6 times longer than the shortest ones, and the shape of which will from the start render it impossible to analyse the results of the experiments. Whole sartorii are unsuitable in such experiments, at any rate when torsion experiments similar to those of Lindhard and Möller (1926—28) are performed, even though these muscles, owing to their shape and structure, are much to be preferred to gastrocnemii. On the other hand, it is extremely difficult to work with isolated fibres, because it is difficult to keep them alive for a sufficient length of time, especially when they are subjected to mechanical influences. Determinations of elasticity are therefore best made on bundles consisting of a fair number of fibres so as to minimise the inevitable effects of the mechanical influences on the individual fibres. Otherwise the preparation should, of course, be selected with due regard to the method which it is intended to employ. In all cases the preparation must be in a state of relative equilibrium when

measurements are made. In any stationary state there exists an equilibrium between the tensions exerted by the different elements of which the muscle is composed. When the equilibrium is disturbed through external interference such as change of intensity of a stimulus, or alteration of the length of the muscle, the different elements composing the muscle (stroma tissue, active and inactive fibres) will tend to attain another state of equilibrium. Such adaption requires, however, an appreciable time for its completion which, among other things, is shown by Gasser & Hill's thermoelastic experiments, and until this has occurred it is, of course, quite impossible to obtain information about the structural elements of the fibre. Changes in friction, viscosity etc., which may be demonstrated during transition from one stationary state to another, are mainly referable to the macroscopic parts of the muscle; they should not be brought into any direct relation to the microscopic constituents of the individual fibre. It must be considered highly probable that the greater part of the elastic afterchanges of the muscle are due to such disturbances of equilibrium. But the fibre, too, consists of a complicated system of microscopic elements, as already mentioned, and their relative positions of equilibrium are also altered when the equilibrium between the macroscopic elements is disturbed, so that if it is desired to obtain more detailed information of the microscopic changes, experiments must be performed with isolated fibres.

As the great majority of the experiments hitherto performed were made with whole muscles, the existing absolute values of the elasticity moduli are quite useless, but the ratio between the elasticity moduli of the resting and the active muscle can be determined with considerable success

on whole muscles, provided that their shape is suitable for the purpose.

The classical experiments in this field are due to E. Weber (1846), who made use of extension experiments and calculated the elasticity modulus from the formula

$$A = 2\,\frac{l_1 - l}{l_1 + l}\cdot\frac{1}{p_1 - p}$$

that is the lengthening of the unit of length on loading with 1 gm. — The experiments showed that the stimulated muscle was more easily stretched than the resting, and moreover that the elasticity modulus of the muscle increases with the degree of extension. These extension experiments were controlled by means of torsion experiments, when it was found that the time of oscillation was greatest in the case of the stimulated muscle, despite the fact that it was both shorter and thicker than the resting muscle.

Objections have been raised against both methods. Against the extension experiments it has been asserted that the elastic after-effects will invalidate the results. Of the older authors Blix especially has opposed this method, and most recently Wöhlisch has drawn attention to the difficulties attending the exact measurement of the muscle length. Thus a sartorius will gradually lengthen appreciably under the load of its own weight alone. Nevertheless, it is possible after a few minutes to obtain a sufficiently constant measurement of the length, especially if the muscles are not too heavily loaded; it is at any rate sufficiently constant if the requirements are limited to comparative measurements of elasticity on stimulated and resting muscles. A number of workers have also been able to confirm Weber's results as far as the extension experiments are concerned. The

torsion experiments employed by WEBER have not been described in detail; but objections have also been raised to this procedure, namely that torsion experiments should not be employed because, normally, the muscle is never twisted in this way. Such objections are, however, irrelevant if the muscle is being considered simply as a physical object. Generally the torsion elasticity gives no information concerning the elasticity in the longitudinal direction, unless, as is the case with muscles, the volume remains unaltered when there is a constant ratio between the two elasticity moduli. Torsion experiments may then be employed; and one of their advantages is that errors arising from measurements of length are abolished.

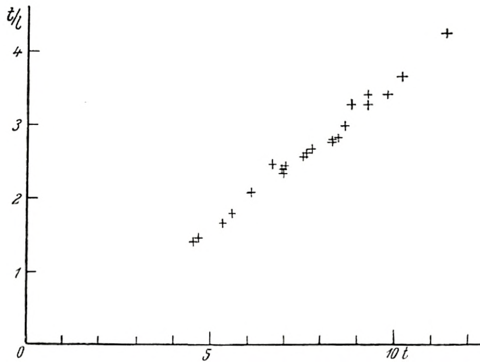

Fig. 13. $\frac{t}{l}$ in arbitrary units as a function of t. t = time in sec.; l = length of bundle. (LINDHARD & MØLLER).

A. V. HILL has objected to the torsion experiments on the grounds that when the muscle fibres are twisted spirally round each other in such experiments, components of force will arise which, irrespective of the elasticity, will cause oscillation of the muscle. According to HILL this would manifest itself by the ratio $\frac{t}{l}$ being constant in the oscillation experiments. LINDHARD & MÖLLER have, however, shown that $\frac{t}{l}$ is not constant, but is a function of t (Fig. 13). If, therefore, under properly controlled conditions there is agreement between the extension and torsion methods, the

results may be relied upon. In the literature a fair number of torsion experiments are reported and in all cases, as far as can be seen, the results confirm Weber's observations. The difficulty in assessing these published results is usually due to the fact that the duration of the torsion but not the length of the muscle is stated; in many cases a statement of whether the muscle was shortened or not will, however, afford sufficient information. Weber has observed that when overloaded to a certain extent a muscle will lengthen when stimulated. This observation, known as "Weber's Paradox" is also reported by other observers. Thus Gad (1879) states that he has seen the writing point fall below the base line during the latent period when a loaded muscle attached to a myograph is stimulated. It is, of course, not necessary merely to overload the muscle in order to elicit the phenomenon. The muscle may be overloaded to such an extent that it fails to appear. Where overloading is sufficiently great the time of oscillation is found to be unaltered on stimulation of the muscle, which is probably due to the fact that the oscillation time of the stroma is involved. Bethe & Happel (1923) by means of special techniques, have obtained corresponding results in certain cases. Steinhausen (1928) employing a modified Bethe's elastometer, has obtained results similar to Weber's. Steinhausen determines the elasticity modulus of the muscle according to the following equation

$$m \cdot \frac{d^2x}{dt^2} + r \cdot \frac{dx}{dt} + ax = 0,$$

in which x is the coordinate of a point in the track of the hammer, m is the mass of the hammer, t the time corresponding to x, r a friction constant, a the elastic antagonistic

force of the muscle corresponding to its starting length. This force can be determined from the following equation

$$a = \frac{Eq}{l},$$

in which q, when the muscle fibres are arranged in parallel formation, may be substituted by $\frac{v}{l}$, in which v is the volume of the muscle. If E remains unaltered during the contraction, t must decrease proportionally to l. This is, however, not so in STEINHAUSEN's experiments; they show that E decreases when the muscle is stimulated. STEINHAUSEN's explanation of this result is that the muscle is perhaps only partially contracting; but it should be noted that whilst a resting muscle is one whose fibres are all at rest, a contracted muscle is one in which many or few fibres are active. Corresponding to a given length a resting muscle has a certain modulus of elasticity, whilst a stimulated muscle of a given length has as many elasticity moduli as it has degrees of stimulation; but all these values are at a lower level than the elasticity modulus of the resting muscle as ascertained by STEINHAUSEN and others.

Apart from some experiments by ERNST and collaborators (1935), based on very doubtful suppositions, there is among experiments on whole muscles only one series reported by GASSER & HILL which is in definite disagreement with WEBER's results. Serious objections have, however, been raised against the technique they employed. GASSER & HILL used a heavy steel spring to which the muscle was fixed at a point "determined by experiment to be optimum". It should have been fixed at the free end of the spring. As pointed out by STEINHAUSEN (1928) the spring does not oscillate about the same point in each experiment so that

its oscillations become highly asymmetrical. The time of oscillation of the resting and the contracting muscle are not, therefore, directly comparable. Moreover, the objection can be raised to Gasser & Hill's technique that the spring may stimulate the muscle by pulling upon it; for both Dreser (1890) and Blix (1891) have observed that sudden loading as well as a sudden removal of loading may produce fibrillary contractions of the muscle. A similar objection may be made to Steinhausen's procedure and, therefore, it appears inadvisable to employ the so-called Bethe-elastometer in future experiments on the elasticity of muscle; but in the present instance possible errors of this nature do not, however, invalidate Steinhausen's results.

Lindhard & Möller have performed experiments with muscle strips of the central part of the frog's sartorius muscle. The preparation employed, as far as possible circular in transverse section, was loaded with 0.363 gm. Stimulation was effected by induction shocks via the myograph and a copper wire, 0.04 mm. thick, which hung from the lower end of the preparation into a beaker of water. The elasticity modulus of the muscle was determined by torsion experiments according to the formula,

$$t^2 = \frac{1+k}{E} \cdot \frac{16\pi l I}{r^4}, \tag{1}$$

in which t is the time of oscillation, k a constant, l the length of the muscle bundle, r its radius, and I the moment of inertia. As the volume of the muscle during the contraction is practically constant we find

$$\frac{l_1}{l_2} = \frac{{r_2}^2}{{r_1}^2}, \tag{2}$$

if we introduce these indices into (1) and at the same time instead of r insert the value determined by (2) we find by division

$$\frac{E_1}{E_2} = \frac{t_2^2}{t_1^2} \cdot \frac{l_1^3}{l_2^3} = \frac{\alpha_1}{\alpha_2}, \tag{3}$$

as $\alpha = \frac{l^3}{t^2}$. In order to determine $E_r = \frac{E_2}{E_1}$ we have only to measure l and t, which can be done with considerable accuracy. In the present experiments l was determined with an accuracy of $\pm$ 0.1 mm.; timing was performed by means of a stop-watch by two observers, which gave an accuracy of $\pm$ 0.1 sec. — As even the slight loading used in these experiments caused a measurable lengthening of the muscle, an attempt was made to correct the length back to l_0 by means of the formula

$$l - l_0 = \frac{k}{E},$$

in which k is a constant. When k is found by other means, the equation enables a determination of l_0 to be made. The

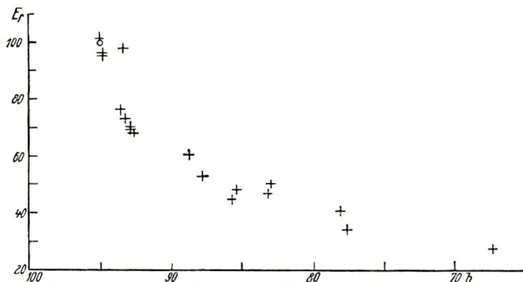

Fig. 14. Ordinate: relative elasticity modulus. Abscissa: length of the contracted bundle in % of the initial length. (LINDHARD & MØLLER).

correction is not exact, but is obviously necessary and its incorporation results in an improvement of the figures

obtained. The damping was not considered, as its influence had to be regarded as being $^1/_2$ per cent. of the time of oscillation. The results of these experiments (Fig. 14) correspond with Weber's results and with the results of experiments previously mentioned, except those of Gasser & Hill.

Quite recently (1935) Sichel has attempted to determine the elasticity modulus on a single resting muscle fibre. This author used the formula $E = \frac{l}{\pi r^2} \cdot \frac{F}{l - l_0}$, measuring l, l_0 and $d = 2\,r$ under the microscope and determining F, the tension, by means of a glass lever previously calibrated. It must, however, be considered doubtful whether this method of measuring tension is satisfactory where the end in view is to obtain exact absolute measurements. Neither can the preparations used by Sichel be considered suitable for the purpose. The author uses fragments of fibres of $^1/_6$—$^1/_1$ mm. in length by stretching them by means of needles, which by his own admission damage the preparation. The maximum extension possible is 15 per cent. Sichel states E for the resting fibre to be 2.5×10^6 dyne/cm^2. In the two published tables the average is 1.56 (0.5—2.8) and 1.57×10^6 dyne/cm^2 respectively; the mean figure stated, therefore, does not appear of much value, less so because the error is not stated, or the number of experiments. These experiments are, however, worth continuing using more suitable methods for the measurement of tension and the degree of extension.

As already mentioned the muscle fibre consists of sarcoplasm and of fibrils, which in turn are composed of short cylinders consisting of alternating anisotropic (A) and isotropic (I) substance. From the measurements referred to previously (Buchthal, Knappeis & Lindhard) the relative elasticity moduli of these two substances can be calculated,

but absolute values can not be obtained because the tension was not measured in these experiments. We may consider that A is contractile, I non-contractile. If we make use of the experiments given in Table VI and compare the unstretched fibre with the extended one, we find, since the tension is the same in the whole of the muscle compartment, e. g.

$$\frac{\frac{2.08 - 1.37}{1.37}}{A_r} = \frac{\frac{1.00 - 0.81}{0.81}}{I_r}, \quad A_r = 0.45\, I_r.$$

On an average we find $A_r = 0.47\ I_r$ where A_r and I_r denote the elasticity moduli of A and I during rest. Correspondingly we find in the case of the contracting fibre $A_k = 0.93\ I_k$[1], where A_k and I_k designate the respective elasticity moduli of the contracting fibre. If we assume on the basis of the existing determinations of elasticity moduli on muscle bundles (LINDHARD & MÖLLER) that the elasticity modulus of the whole muscle compartment is less during contraction than at rest, it follows from the figures above that I_k is about $\frac{1}{2}\ I_r$, while A_k is about equal to A_r. The results of the above elasticity determinations cannot be used quantitatively here; but an examination of all existing measurements indicates that the decreased elasticity modulus of the whole muscle compartment during contraction will more readily be produced by a change in the elasticity modulus of the extended I-substance than by a change in the modulus of the greatly shortened A-substance. A further decrease in the A-substance would otherwise imply an improbably large decrease in the resting length. As the most probable result it must, therefore, be concluded that:— In the resting fibre

[1] This figure is less reliable than the resting value as the changes in length and elasticity modulus may not necessarily run parallel.

the elasticity modulus of the *I*-substance is about twice as great as that of the *A*-substance, whilst during contraction the two moduli are nearly equal, for on stimulation the elasticity modulus of the *I*-substance is reduced to about half the resting value, whilst the elasticity modulus of the *A*-substance remains unchanged. A possible explanation of this apparently uneconomical phenomenon appears to be that the decrease of the elasticity modulus will act as a "buffer" in the case of sudden, strong, mechanical stresses. At the same time it must be admitted that the assumption that *I* is non-contractile is open to question in so far as a slight contractility of *I* might be disguised by contraction of the stronger *A*-section.

The breaking strain of muscle tissue determined in extension experiments with whole muscles is only of slight physiological interest, because it must depend chiefly on the muscle stroma; at any rate it is impossible to draw conclusions from such experiments regarding the elasticity of the muscle. Carvallo & Weiss (1899) have examined muscles in situ with intact circulation in guinea-pigs and frogs. The authors find that frog's muscles are generally torn by a traction of 6000 gm. per cm.^{2}, a little more for stimulated muscles than for resting muscles, which is attributed to "the counter-traction of the contracting fibres against the loading". The limit of elasticity, on the other hand, is a quantity which, especially in experiments with isolated bundles and single fibres, is of some importance in the estimation of the results of such experiments. Hitherto a good deal of such work has been in vain because due regard was not paid to the existence and importance of physiological limits.

Much earlier work was performed in order to determine the shape of the curve of extension, whereas subsequent

experiments have aimed at determining the tension-length diagram and then have shown that the *stretch- and release*-curves of the innervated muscle did not coincide, so that the latter therefore could not be considered to be "a new elastic body". None of these experiments have given satisfactory results because the necessary conditions for such experiments have not been observed, and because different workers have taken widely differing views of their material. A number of experiments by H. H. WEBER, performed with a view to comparison between the curve of extension of a whole muscle and that of a myosin thread, are especially open to such criticisms. Such analogies are now unnecessary, since we are able to determine the stretch-curve of the single sections of the muscle fibril. Further discussion of tension-length experiments on whole muscles will not be considered here; reference will only be made to a series of experiments by ASMUSSEN (1936) performed on bundles and single isolated fibres. A close examination of these experiments will show their superiority over similar experiments on whole muscles.

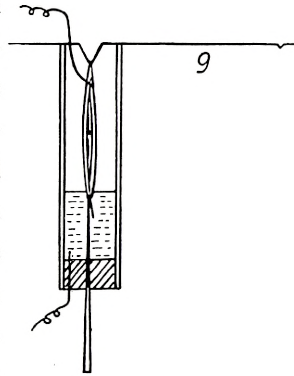

Fig. 15. Device for recording the tension of single fibres or small bundles. g = glass rod. (ASMUSSEN).

In this experiments ASMUSSEN employed the semitendinosus muscle of the frog, a muscle that is wellsuited for such experiments, because its fibres are comparatively short, very nearly cylindrical, and of the same length as the bundle, so that they can be fixed by means of the tendinous tissue to which they are attached, without injury to the fibre substance. The arrangement of the experiment will appear from Fig. 15.

The tension measurements were made with the aid of a glass lever, previously calibrated. This method is obviously of limited accuracy, but that the results obtained bear some relation to the conditions really existing, is shown from the fact that measurement of the area of the tension-length curve for contraction within physiological limits gives the expression $\frac{1}{5}$ *Tl* for the potential energy, where *T* is the isometric tension and *l* the resting length; a value corresponding very well to the one stated by A. V. Hill. Thus the accuracy is at any rate sufficient for the experiments discussed here. The difficulties in obtaining exact results in muscle physiology are as a rule associated not only with the apparatus but with the preparation also, so that in many cases the limits of accuracy of the experiment will be determined, less by the perfection of the apparatus, than by the condition of the preparation.

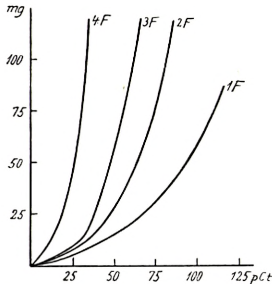

Fig. 16. Length-tension diagram of small bundles (1—4 fibres). Ordinate: tension in mg.; abscissa: length in % of resting length. (Asmussen).

Passive extension of one or a few muscle fibres will result in curves such as are shown in Fig. 16, where the abscissa represents extension in per cent. of the resting length, the ordinate representing the tension in mg. As will be seen the curves are not straight lines but are chiefly of the shape seen in extension experiments on whole muscles. This shows that the shape of the curve of extension of whole muscles within physiological limits is due primarily to the fibres, and that the connective tissue stroma becomes of importance only on more marked extension. This shape of the curve moreover explains why Sichel, who works with

the lowest and least reliable part of the curve, arrives at the result that the curve of extension is a straight line. When experiments are performed, as are those of SICHEL, with short, more or less damaged fragments of fibres, where the initial length is in doubt and the extension amounts only to 10 per cent. of the latter, the result must be unreliable; for the shape of a curve cannot be determined on the basis of so short a portion of the whole. ASMUSSEN moreover finds that the curve of extension of a resting fibre or bundle is completely reversible up to a degree of extension of a little more than 40 per cent. If this length is exceeded the curve is no longer reversible, but the curve of relaxation will lie at a lower level than the curve of extension. The author concludes from this that the limit of elasticity of the fibre must lie at this degree of extension. That this conclusion is correct will appear from the histological measurements of BUCHTHAL, KNAPPEIS & LINDHARD, which show that the histologic structure of the muscle fibre, especially the cross-striation, disintegrates at a limit lying at about 150 per cent. of the resting length. This limit is not sharp and cannot be so, because a fairly considerable individual variation will always exist, so long as we have no exact criterion of the physiological condition ("Vollwertigkeit") of the fibre. The wider the units with which we work, the more does this apply, because all the fibres of the bundle are not necessarily in the same condition or extended to the same degree in any given experiment.

If then we consider the stimulated muscle fibre it can be seen that it is a new elastic body with a shorter length of equilibrium and lower elasticity modulus than the resting fibre. If the stimulated fibre is prevented from attaining its new resting length a tension will be produced in it. Owing

to the rapid course of the contraction it is theoretically impossible to determine the extension curve corresponding to a single stimulus. Therefore it becomes necessary to work with tetanized preparations. There are then two possible procedures to choose; either the tetanized bundle can be stretched, or the bundle previously extended, can be tetanized. So long as we remain within physiological limits the results will in the main be identical, but if these limits are exceeded the result will become different in the two cases as may be expected.

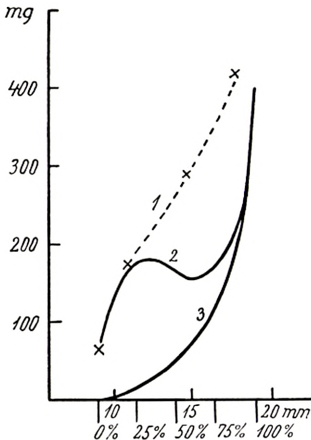

Fig. 17. Length-tension diagram. Small bundle of 12 fibres. 1. stretched during contraction. 2. stretched and then stimulated. 3. resting fibre. Abscissa and ordinate as in Fig. 16. (Asmussen).

If the muscle, extended beforehand, is stimulated we shall obtain a tension-length diagram of the shape shown in Fig. 17. As will be seen it corresponds fairly closely to the curves arrived at by experiments with whole muscles (Sulzer and others). In different experiments the point of the curve will occupy somewhat different positions depending on the shape of the fibres and their individual lengths as well as on the general condition of the preparation. This type of curve, whose shape has never been explained, has been used in attempts to prove that the stimulated muscle cannot be considered a new elastic body. However, when the change in excitability of the muscle produced by stretching is taken into account, such arguments cannot be sustained. Clinical investigations of Brinch-Eliassen (1925) suggest

that the threshold value of the stimulus is lower when the muscle is relaxed than when it is passively extended. If the threshold value of the direct stimulus is determined for a muscle bundle, the result shown in Fig. 18 will be obtained. The curve shows that when the extension reaches about 25 per cent. of the resting length the threshold value of the stimulus begins to rise, slightly at first, but gradually more and more until at an extension of 50—60 per cent. of the resting length, the excitability disappears. If the stimulus is not of too long duration nor forced to extreme limits, the change in excitability is reversible. The bundle will resume its original resting length, and regain its former excitability and corresponding tension. The changes of excitability referred to here do not appear when indirect stimulation is employed, because the stimulus proceeding from the end-plate is at a higher level than the threshold value; therefore the effect of an indirect stimulus will remain almost unaltered until, at an extension of about 60 per cent., it suddenly disappears. When this point is reached the bundle will behave as though unstimulated.

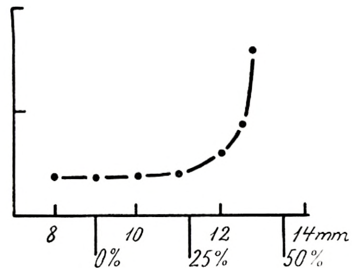

Fig. 18. Excitability of the directly stimulated fibre with increasing stretching. Ordinate: threshold in arbitrary units; abscissa: stretch in % of resting length. (ASMUSSEN).

If, on the other hand, a resting bundle is stimulated and then, under constant stimulation, passively extended, the conditions become so complicated as to be beyond our control. To begin with, the muscle will assume its shortest length of equilibrium; but gradually as the extension continues stimulation becomes less effective and simultaneously

there will occur, as shown by various experiments of Asmussen (1936) and others, a slow regression of the change of elasticity. Since the tetanic curve is formed by the summation contractions of curves of single fibres this will cause the tension to be added to an existing "residual tension" (tension remainder) so that the resulting curve will be at too high a level. The curves arrived at by these two different procedures will, therefore, coincide only in their first part. (Fig. 17.) This does not imply, however, that the stimulated muscle is not a new elastic body, but it means, as has already been pointed out, that the active muscle when stimulated at different lengths is transformed into different elastic bodies. From this point of view it appears inadmissible to employ the term "force of contraction", or similar terms when referring to the difference between the tension of the resting and the contracted muscle corresponding to a given length.

It is assumed that stretch and release of the bundle must take place so smoothly that possible displacements between the individual fibres, relative to each other, will soon be evened out. If the contraction or the relaxation takes place suddenly it will be impossible to obtain smooth tension-length diagrams, for a sudden jerk may act as a stimulus, and possible displacements cannot pass off until some time after the movement has ceased. The same applies to an even greater degree when whole muscles are used. On the other hand it will be impossible to work exceedingly slowly, for then further complications will occur owing to the onset of fatigue. For these reasons, therefore, it is impossible to construct a tension-length diagram representing the theoretical working maximum of the muscle.

Stimulation of the Muscle Fibre.

In the preceding chapter it has been shown that the resting, unstimulated, muscle fibre is a body possessing certain elastic properties, and the stimulated fibre a body with certain other elastic properties. When it is undisturbed by stimuli, the resting state is relatively stable. On the other hand, the fully developed state of contraction is labile, so that even under the most favourable conditions it can only be maintained for a very short time, a few seconds at the utmost. The apparent difference in the case of whole muscles is due to the fact that in this case interference phenomena are mostly being observed. Despite the variations which may be observed experimentally in these two conditions, there will always exist a characteristic difference between them, for the stimulated fibre has a shorter length of equilibrium than the resting fibre. Within physiological limits both the resting and the stimulated fibre will behave in general like elastic bodies. Especially characteristic of muscle fibres is the fact that, under the influence of a stimulus, the resting fibre will pass into a different elastic state and then, after stimulation wears off, will return through a stage of restitution to the resting condition. During the transition from one elastic state to another the fibre will pass through an active state, the actual stage of contraction, in which it will be able to perform work under suitable external conditions. This, however, must not be considered to be some special property inherent in the organ, but is a direct consequence of the characteristic elastic property of the muscle. During the "process of contraction" the same things happen in the fibre as in a stimulated muscle which had been passively extended to the equilibrium length of

the resting muscle and then released. The contracted muscle taking up its equilibrium length is no more active than the resting muscle. The activity of the muscle may be considered to be of the same nature as the activity of the rubber cord, both phenomena being simply the consequence of an elastic body having been disturbed from its position of equilibrium.

With gradual increase in our knowledge of the events taking place in the muscle fibre as a result of stimulation, and with extended understanding of muscle function, we are faced more and more with difficulties arising from the inadequacy of the terminology at our command. Thus, to describe a whole series of, apparently, highly specialised phenomena we have only the single term: contraction. This term must, for example, serve to describe the shortening of the thick end, and the elongation of the thin end of the conical muscle fibre; its use results for instance in such linguistic monstrosities as "isometric contraction" and "excentric contraction", and the terms "static" and "isotonic" contraction. This incomplete terminology only serves to reflect the corresponding confusion which exists as regards definitions and ideas of muscle function. The phenomena taking place in whole muscles, which in part must be related to the conditions of stimulation and the architecture of the muscle, have been and still are confused with the phenomena of the individual fibre, whose shape and innervation must likewise influence the ultimate result of the process of stimulation. Finally these processes are in turn confused with those taking place in the single muscle compartment, the essential unit of muscular activity.

An attempt will be made here to give an account of the process of stimulation in the single fibre under different conditions, whilst the mechanism of muscular activity itself,

so far as it is known, will be discussed in a subsequent chapter. In most cases it is impossible to follow the process of stimulation in the muscle than by observation of the effects it produces, so that it may become necessary even here to refer to muscle tension etc.; but where the description of a highly complicated phenomenon is described under several headings, a certain amount of repetition is unavoidable.

The Stimulus.

The muscle fibre may be stimulated directly or indirectly. The physiological stimulus is, however, always indirect; that the somatic motor nerve branch also may be influenced by artificial stimuli is of no importance in the study of muscle function proper. There is general agreement on these elementary points. A great deal of confusion in this field has resulted from badly arranged experiments and ill-controlled conditions, in consequence of which indirect stimulation has taken place very frequently when it was believed that a direct stimulus had been applied.

The muscle fibre can be directly stimulated by mechanical, thermal, chemical, and electrical means. The latter has, however, mainly been employed hitherto in experimental work as it is difficult in the case of the first three forms to adjust the strength, to limit the time of action, or to employ them in such a manner that they do not damage the fibre. Provided the electrical stimulus is not made unnecessarily strong or continued beyond a certain time, depending empirically on the conditions of the particular experiment, it will cause no injury to the fibre, which may indicate that it is of similar nature to the natural stimulus. Therefore, it may be supposed that the results obtained by direct and indirect electrical stimulation are comparable.

According to the classical theory of the physiological process of excitation, a nerve impulse from some ganglion cell, arrived at the motor end-plate and was "transmitted" in some way to the "contractile substance" of the muscle fibre, causing this to become active, "the state of contraction". Since muscle physiology is concerned only with the subsequent processes, it is of no consequence whether the nerve impulse is produced by natural processes, or by an artificial stimulus applied to the peripheral nerve. At the point of entry of the nerve, the so-called "nervous equator of the muscle", the arrival of the nerve impulse produced a "wave of contraction" which was propagated at a fairly slow rate towards the ends of the muscle.

This conception was ultimately elaborated about 1870 as the doctrine of the so-called "action current". Even though this is now of historical interest only, at least so far as muscle is concerned, nevertheless it played so important a role in the investigations into muscular function as to merit its brief mention here, if only for the reason that discussion of this question directed attention also to the part played by the motor end-plate in the natural excitation of muscle.

When two non-polarizable electrodes were placed some distance apart on an undamaged muscle and connected with a galvanometer a deflection of this would be observed when the muscle was stimulated electrically. This deflection would appear as a diphasic curve and was termed the "action current".

The phenomenon was carefully studied by Hermann, who explained it as due to a "wave of contraction" in the muscle which first passed under one conducting electrode, whereby a deflection of the galvanometer occurred in one

direction, and then under the other electrode, causing a deflection in the opposite direction. HERMANN, however, soon modified his account to the effect that it was not the wave of contraction itself which gave rise to the electric phenomena, but that the latter were associated with the process of stimulation, as any part of the muscle when excited became negative to a resting part. In this manner a "wave of negativity" occurred, which advanced over the muscle at the same rate as the "wave of contraction".

Many years later the doctrine of the "action current" was revised by PIPER (1912), who worked with improved technique, including EINTHOVEN's string galvanometer. PIPER was fully aware of many of the difficulties involved in the support of HERMANN's theory but had no other explanation to offer. PIPER moreover extended the existing data by experiments on humans, some with voluntary muscle function, some with application of an artificial stimulus to the nerve trunk. In these experiments both stimulation and leading off of action currents took place percutaneously, and groups of muscles were employed. The curves so obtained were in complete correspondence with those obtained from isolated muscles, but the difficulty of the theoretical explanation of the action current was increased. As recently as 1925 FULTON has repeated the early theory quite uncritically, and no further facts of importance have since appeared.

The first real opposition to the theory of the action current which PIPER's work supported came from TSCHIRJEW (1913), who pointed out that by leading off transversely from a muscle, action currents of the usual form were obtained, which was unexplained by the theories then in vogue; he considered the action currents to be artefacts.

The validity of Hermann—Piper's hypothesis was again questioned by Henriques & Lindhard (1920), who criticised some of Piper's hypotheses and raised a number of anatomical as well as functional objections to the theory. We shall not revive the discussion here but only emphasize certain important points. Henriques & Lindhard maintained that the innervation of muscle is of such a nature as to preclude the possibility, in the whole muscle of the occurrence of a progressive wave-motion of the kind required by the theory, particularly in the case of groups of muscles (cf. for instance Fig. 19 reproduced from Bardeleben's Handbuch). Thus, even in a small muscle bundle we find not one, but many points of entry of the nerve branches (see Fig. 19), which excludes the possibility of a "wave of negativity" proceeding in the same direction in all fibres. Henriques & Lindhard found that in certain cases action currents could be obtained in the absence of muscle contraction, and that under other conditions powerful contractions could be obtained without action currents; e. g. the curarized muscle apparently produced no action current on direct stimulation. They showed, moreover, that the two phases of the diphasic action current were not invariably symmetrical, which was considered to be in contradiction to the theory. From their experiments Henriques & Lindhard concluded that Hermann—Piper's theory was untenable; moreover that the action current must be considered to be connected, not with the substance of the muscle fibre, but

Fig. 19. Distribution of nerves in the flexor group of the fore arm. (Frohse & Fränkel).

with the motor end-plate. This view aroused much criticism, which was especially centred on the behaviour of curarized muscles, as it was claimed that curarized muscles gave as powerful currents of action as did non-curarized muscles. The experiments were later resumed by LINDHARD (1932) with similar results to those previously obtained; and experiments by ASMUSSEN (1935) and BUCHTHAL & LINDHARD (1934—35—36—37) and HÖFER (1934) have now suggested that "action currents" originating from curarized muscles may be due either to defective curarization or are artefacts due to spread of stimulus. ("Reizeinbruch", c. f. SCHÄFER (1936)). In such work experimental errors are easily made, since the stimulus used to obtain the mechanical record may easily interfere with the arrangements for recording action currents. If care is taken, it is, however, possible to prevent this, not only on fresh tissues but also on curarized or paralysed muscles. The chief difficulties are those referred to above. As to curarization, it is a wellknown fact that curare is an inconstant and difficult drug with which to work. In view of this difficulty ASMUSSEN (1935) has systematically examined different methods of curarization and has shown that the effect varies greatly; but by far the most serious difficulties are those caused by the stimulation. Working with indirect stimuli we can easily prevent stimulus "escape", and ascertain whether this requirement has been fulfilled or not; but working with direct stimulation, which in the case of curarization is unavoidable, we have no effective control with regard to spread of stimulus. The only criterion lies in the result itself. If the galvanometer gives no deflection at all, there has been no spread of stimulus and then there is no "action current". Care is necessary in order to achieve this result. First, large frog gastrocnemii may be

employed, so as to ensure that the leading-off electrodes, which are placed on the lower third of the muscle, shall be as far away from the stimulation electrodes as possible. This is essential, because the threshold for direct stimulation is far higher than for indirect. Again, the fibres may be stimulated in the transverse direction of the muscle, and one of the stimulating electrodes may be earthed. Lastly, portions of the muscle where a large nerve branch approaches the surface, should be avoided; but in this respect individual differences may complicate the conditions of the experiment. The experiments are easily performed on large gastrocnemii, but are far more difficult with sartorii. The reason may be that the paths taken by the stimulating current are more or less confined to the comparatively large muscle mass in the upper part of the gastrocnemius, whose fibres, and blood capillaries also, follow an oblique course and are not directed towards the leading-off electrodes. In the strap-like sartorius which may be preferable from another point of view also, the reverse is the case, for all the conducting elements pass in the same direction from the point of stimulation to the conducting electrodes.

In a subsequent publication Henriques & Lindhard (1923) emphasized that it is only possible to determine the frequency of the action current when the curve is regular and shows no signs of interference. To attempt, as Piper has done, to distinguish between "major and minor waves" ("Haupt- u. Nebenwellen"), is valueless. In the case of very vigorous and thus comparatively short voluntary muscular contractions it is possible from a group of muscles such as the flexors of the forearm to obtain smooth curves, the frequency of which lies between 40 and 65 per sec., which was supposed by Piper (1912) to be the frequency

with which the motor centres sent out their impulses. This smooth rhythm can only be demonstrated in the case of maximal contractions. It cannot be decided with certainty whether the rhythm of the action currents corresponds to the rhythm of the ganglion cells or not, even if the rhythm of the action current follows the rhythm of an artificial stimulus.

The whole of the question of the essential nature of the action current and its possible importance in the organism was reconsidered when LINDHARD (1924) revived the old "Discharge Theory" (Entladungshypothese). HENRIQUES & LINDHARD's paper (1920) made it evident that in some way the electrical phenomena must be associated with the motor end-plate and, therefore, a comparison was suggested between this organ and the electric organs of certain fishes. It soon became apparent that we possessed so complete a literature on the subject that it could be approached both ontogenetically and histologically. In the case of one of the strong electric forms, however, the question is still unsettled. This is the Malapterurus, whose electric plate is associated with the skin and does not seem to be developed from musculature. The young of this fish have, however, never been found, despite very careful search. In the Malapterurus the whole of the electric organ, which is capable of producing an E. M. F. of about 250 volts is innervated from two large (macroscopic) nerve cells in the central nervous system, whilst in all forms where it is developed from skeletal muscles it is innervated from the somatic motor nerves attached to the latter. The correspondence demonstrated between the electric plates and the motor end-plate is so great that they must be considered morphologically identical organs and, therefore, the electric "shock" of the fishes in question was considered to be a phenomenon analogous with the "action

current" of the muscle. This concept was further supported by experiments on Astrape japonica of Fuji, who employed an oscillograph and photographic recording, and examined the electric discharges from a portion of the electric organ of this fish. He obtained a curve which bore a striking resemblance to the so-called single-contraction curve of a skeletal muscle. After a latent period (7.6 σ after the commencement of the stimulus) it rose comparatively abruptly to a maximum, then fell again more slowly to the base-line. Finally there was a slight, secondary rise in the curve (Fig. 20). The curve conforms to the equation

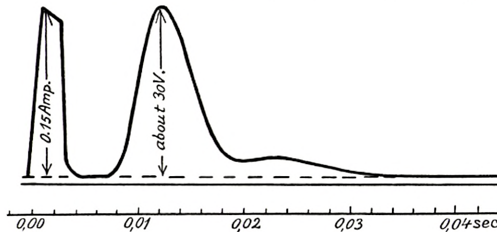

Fig. 20. Stimulation and response of electric organ of Astrape japonica. (Fuji).

$$y = Ae^{-b^2 \log^2 \frac{x}{x_0}},$$

which has been derived from the equation for the exponential curve of errors. On the basis of his analysis of the curve Fuji believes that the shape of the curve is due to the fact that not all the plates react simultaneously but in succession, the beginning of the curve corresponding to the plate first reacting, whilst its maximum represents the greatest number of plates reacting simultaneously. According to Fuji the secondary rise is due to the discharge of the last reacting plates acting as a stimulus on the first plates, the reaction of which by that time has already passed off.

If this is correct it would be expected that the first phase of the curve of the action current should correspond to the above equation. That there might possibly be some basis for this, would appear from Fig. 21 (Lindhard, 1931), in which a number of ordinates calculated according to Fuji's formula are inserted into the first phase of the curve

of an action current obtained by KEITH LUCAS with a capillary electrometer. The slight deviation towards the end is possibly due in part to the same cause as the deviation in FUJI's curve, and partly also to the commencement of the second phase of the action current. The so-called single-contraction curve behaves in a similar manner; in this case, however, the correspondence is not so close.

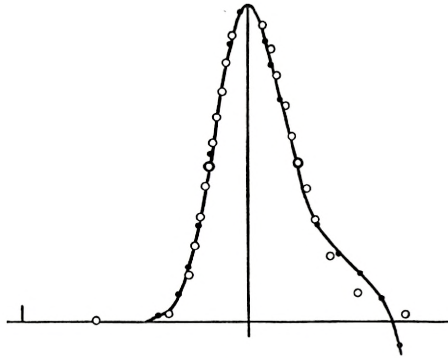

Fig. 21. First phase of action current from a frog's muscle. Circles calculated from FUJI's formula.

As the interference curves obtained from experiments on whole muscles are always difficult to interpret, ASMUSSEN & LINDHARD (1933) tried to examine the action current of single muscle fibres. Similar experiments had already been made by GELFAN & BISHOP (1933), who stimulated fibres of the frog's membrana basihyoidea mechanically. When such stimulation was applied to the fibre substance no action current appeared in spite of the occurrence of a localised contraction, though it was seen occasionally when, as the authors stated, a nerve branch had been stimulated. ASMUSSEN & LINDHARD did not employ frog's muscles but lizard's muscles (Lacerta agilis), which have the advantage of possessing localized end-plates. The superior, subcostal part of the M. obl. abd. int., which consists only of a double layer of fibres, is used, for which reason it is easy to observe end-plates in the fibres by transmitted light. In this muscle the fibres are 13—14 mm. long and 40—70 μ thick. The end-plate is of an almost circular shape with a diameter of

30—60 μ. Examination and stimulation was performed under a binocular microscope with a magnification of about ×125. As it became at once apparent that both electrical and thermal stimulation affected the galvanometer the motor end-plate itself was mechanically stimulated by means of an extremely fine glass needle, which like the conducting electrodes, was adjusted in position by means of Péterfi's micromanipulator. Mechanical stimulation is not consistently effective, at any rate when used with caution so as to avoid damaging the preparation. To remedy this the preparation was sensitized to such stimuli by adding a minute amount of a strychnine or phenol solution to the drop of Ringer's solution in which it was immersed. The action currents were led off through a condenser-coupled amplifier to a string galvanometer (Boulitte), by means of fine platinum electrodes. The amplifier recorded alternating currents down to a frequency of 5 per second. For further details reference may be made to the original paper.

When both electrodes were placed on the muscle fibre and the latter was stimulated mechanically close to one of the electrodes no deflection appeared on the galvanometer, even when a local contraction of the part stimulated could be distinctly observed under the microscope. As will be shown later, this does not necessarily imply that the process of contraction in the fibre does not give rise to electric phenomena; these, however, could not be demonstrated by means of the apparatus described above. When, on the other hand, one electrode was placed on the fibre and the other immediately at the edge of an end-plate (it could not be placed on the end-plate, as it would then have interfered with the mechanical stimulation) and the end-plate then was irritated, a diphasic deflection of the galvanometer was

obtained as shown in Fig. 22. When one electrode was situated at the end-plate, the other could be placed on the same fibre or a neighbouring fibre, and moreover the muscle fibres on either side of the end-plate could be cut through by means of a fine needle, and the end-plate thus comparatively "isolated", all without changing the form of the electrical response to any considerable extent. As will be seen from Fig. 22, most of the curves are uniformly diphasic; but in a certain number of cases forms like those shown in Fig. 22 left are seen, different from the usual type though the conditions were apparently the same and there was no injury produced. A direct comparison between these curves and those obtained from experiments on whole muscles is of course difficult, because the latter must be considered an expression of interference phenomena. All things considered, it seems likely that the first phase of these curves is identical with the first phase of the action current. The second phase of the electrogram is no more symmetrical with the first than in the curves from experiments with whole muscles; and, therefore, as already pointed out by HENRIQUES & LINDHARD (1920), it is improbable that the two phases are due entirely to one and the same underlying process. The second phase of the action current, as it appears in the measurements made here, might be connected in some way with the first, rapid change in the contraction potential (BUCHTHAL 1934) referred to later. If this is so, the second phase of the action current thus has its origin partly in

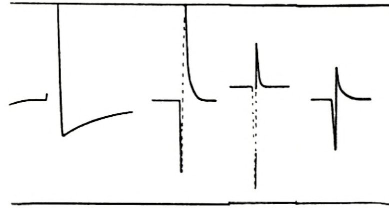

Fig. 22. Action currents from single muscle fibres (lizard). Direct mechanical stimulation of the end-plate. (ASMUSSEN & LINDHARD).

processes in the fibre-substance. Asmussen & Lindhard's failure to demonstrate electrical changes on stimulation of the fibre itself must be due to the fact that the mechanical stimulus employed by them could only produce localised contractions of the fibre.

There are, however, further difficulties involved in such a comparison, first and foremost that Asmussen & Lindhard's apparatus was entirely different from that used by Buchthal and Lindhard (see below). This does not imply, however, that the above explanation of the second phase of the action current is completely inadequate. It is worth emphasizing in this connection that the potential changes both of excitation and contraction, are monophasic and that their time relations appear to correspond to those between the two phases of the action current; but the time relations of the contraction potential and the second phase of the action current do not correspond, which may have some connection with the type of amplifier used by Asmussen and Lindhard.

Finally, it may be pointed out that the ratio between the number of the usual diphasic curves and the "monophasic" ones is about the same as the ratio between the cases in which the changes in excitation and contraction potentials are opposite, and those where they are in the same direction.

Owing to the experimental difficulties previously referred to, it can only be deduced from these experiments that there is a potential difference occurring when the end-plate is stimulated which cannot be observed on direct stimulation of the fibre.

Accordingly, it appeared probable that the rapid diphasic oscillation was, as supposed by Lindhard (1924), in some

way associated with the process of excitation in the muscle fibre; this gives, however, no further insight into the mechanism of excitation. The next step towards the understanding of this was taken when measurements of current were replaced by electrostatic measurements, which have

Fig. 23. Lizards muscle bundle, living unstained fibres. Upper electrode on motor end-plate, lower electrode on the same fibre. Magnification 450 ×. (BUCHTHAL & LINDHARD).

been introduced into this field by BUCHTHAL. A brief survey of the principle of this method will be found in a following chapter. For further details the original papers may be consulted.

After BUCHTHAL & PÉTERFI (1934) had shown that in frog's muscle fibres there exists a potential difference between different parts of the surface, increasing with the distance apart of the electrodes, i. e. with the number of

muscle compartments included, BUCHTHAL & LINDHARD (1934) measured the potential difference between the motor end-plate and the fibre substance by means of the same technique. For histological reasons these experiments were made with lizard's muscles. The micro-electrodes were placed 40—60 μ apart on the same fibre; the grid electrode was placed on the motor end-plate (Fig. 23). It then appeared that whilst the potential difference measured on the fibre amounted to 0.5—1.0 mV., the potential difference between fibre and end-plate, measured with the electrodes the same distance apart, was about 10 mV. Moreover, the fibre-fibre potential difference in some cases went in the same direction, in several cases however in the reverse direction, to the fibre-end-plate difference. (Fig. 24.)

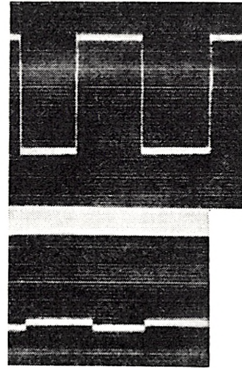

Fig. 24. Potential difference between motor end-plate-muscle fibre (upper curve) and between two points on the same fibre (lower curve). Electrodes same distance apart. (BUCHTHAL & LINDHARD).

The measurements of currents previously dealt with may give some indication that the potential difference as measured electrostatically plays a part in the excitation process. Therefore BUCHTHAL and LINDHARD (1936) examined the effect of temperature changes upon the fibre-end-plate potential difference, at first within the limits at which laboratory experiments usually take place; later it was believed that by extending the temperature range (still within physiological limits) a clue might perhaps be obtained as to the processes underlying the production of the potential difference. It appeared from these experiments that the fibre-end-plate potential difference, like the fibre-potential (which will be

dealt with in detail in a subsequent chapter) varied directly with the temperature (cf. Fig. 25). Calculation of Q_{10} gave 1.8 ± 0.08 with an average error of 23 per cent. — But there was no significant relation between Q_{10} and the general level of the temperature curve. In these experiments it was, however, striking that the fibre-end-plate potential difference[1], which often remained unaltered for at least 1 hour at a constant temperature, was unable to withstand considerable changes in temperature. Furthermore, it appeared

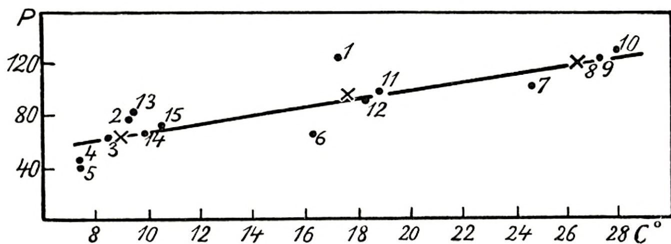

Fig. 25. Relation between end-plate-fibre potential and temperature. Numbers indicate sequence of measurements; ordinate: potential in arbitrary units; abscissa: temperature.
(Buchthal & Lindhard).

that even moderate rises of temperature might easily cause alterations of potential to become partly irreversible.

Buchthal & Lindhard (1935) have also examined the effect of curare and radium emanation on the F—E potential. Lizard's muscles were used and measurements were made by Buchthal's electrostatic technique, recording with an electron tube voltmeter. It was first ascertained that the above potential differences existed in the resting system, and it appeared once more that the F—E potential was nearly 10 times as large as the F—F potential with the same distance between the electrodes in each case. The potential difference

[1] In the following termed the F—E potential, the potential difference between two parts of the same fibre being termed the F—F potential.

in different animals often varied in size and direction, but the variations found in preparations from the same animal were small and may be due partly to a different position of the end-plate within the fibre. Although in $^2/_3$ of the cases examined the end-plate was positive in relation to the fibre, the reverse was the case in the remaining $^1/_3$. No satisfactory explanation can be offered for this fact at present, even considering the possibility that the location of the electrode in relation to the end-plate may have some effect on the direction of the potential as indicated by experiments on amoebae made by BUCHTHAL & PÉTERFI (1937).

The principal results of the experiments with curare are shown in Table VII. The greater changes found in the *F—F* potential are chiefly due to a different distance between the electrodes.

As will be seen the curare effect consists of a reduction

Table VII.

Fresh preparation potential		Curarized preparation potential	
F—E	*F—F*	*F—E*	*F—F*
—96	—18	— 6	— 7
+40	—16	— 6	— 4
+31	+ 7	—27	—28
+17	—16	+24	+24
—80	+ 2	— 3	— 3
+95	— 5	— 4	— 4
—56	— 4	—13	— 4
—65	— 5	+20	+22
+33	— 3	+12	+18
—74	+ 8	+ 6	+ 5
—75	—18	+20	+ 7
—78	—11	+ 6	+ 8
—33	+ 5	+20	+12
+70	+ 6	..	..
+38	+ 7	..	..
+78	+ 2	..	..

of the high F—E potentials to the order of magnitude of the F—F potentials. The first experiments were performed by measuring the F—E potential of a preparation, covering it with a 1—3 per cent. fresh curare solution and then, after 30—60 mins., when the whole muscle was poisoned, again measuring the F—E potential of the same end-plate. As a

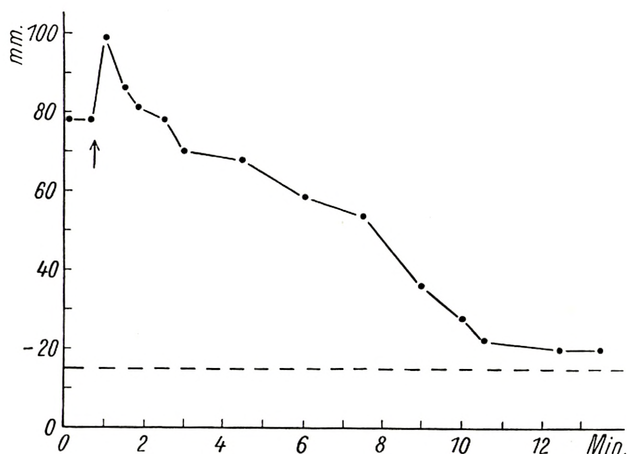

Fig. 26. Effect of curare on the potential difference between end-plate and muscle fibre.
- - - - - -: potential difference in fibre.
↑: application of curare.
Ordinate: potential in arbitrary units.
Abscissa: time in minutes. (Buchthal & Lindhard).

control the F—E potential was measured twice on unpoisoned preparations at the same intervals of time as in the poisoned ones.

Besides these preliminary experiments a number of others were performed, in which the course of the process of curarization was followed. The F—E potential was first determined in the usual manner, and a curare solution then run slowly on to the preparation, the potential difference being read simultaneously at frequent intervals; by means

of a number of varied control experiments we made sure that the addition of the curare did not in itself produce disturbances of potential that would influence significantly the experimental results. The experiments indicate that the *F—E* potential steadily diminishes, sometimes after a short initial rise, until the *F—F* potential has been reached (Fig. 26). If the F—F potential has the reverse sign of the *F—E* potential, the latter must thus change sign in the

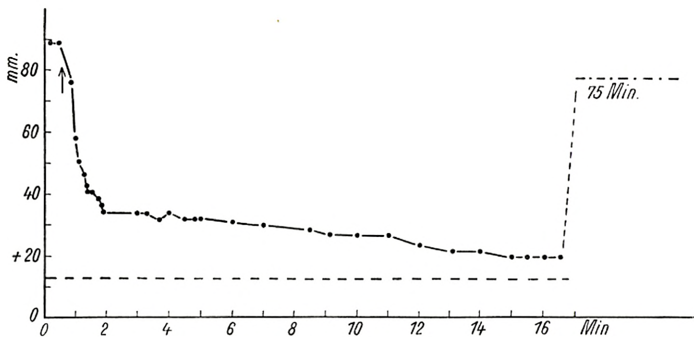

Fig. 27. Effect of curare on the end-plate-fibre potential (see Fig. 26). —·—·—·—: potential difference after removal of curare. (BUCHTHAL & LINDHARD).

course of the curare action. When curarization is complete the fibre is no longer excitable by indirect means but can still be stimulated directly[1]. If the curare is removed by rinsing with Ringer's solution, the *F—E* potential will be reestablished and simultaneously it will again be possible to stimulate the fibre indirectly. (Fig. 27.)

These experiments show that the *F—E* potential is a necessary condition for indirect excitability; when this potential difference has been abolished the end-plate is

[1] In this connection it may be emphasized that the threshold value both of mechanical and electrical stimuli is considerably higher with direct than with indirect stimulation. With electrical stimulation the effect is independent of the direction of the current.

simply a continuation of the electrode transmitting the direct stimulus. Moreover, in correspondence with Asmussen's experiments, these results show that, as regards the time course of its action, curare is a very inconstant drug, and also that the mechanical recording of the muscular contraction cannot be employed when we want to make certain that all the end-plates of a preparation are paralyzed. This can only be secured in preparations that can be controlled by observation under the microscope. Whole muscles are, therefore, quite unsuitable objects on which to examine the curare effect, and such experiments reported in the literature must be regarded with scepticism, especially where the object has been to examine the electrical changes in muscles.

As is well known, emanation emitted by radioactive substances is able to ionize gases and fluids, and is moreover able to influence boundary surfaces of living tissue and their permeability to ions. Buchthal & Lindhard (1935) have examined the effect of the radium emanation on the F—E potential difference. The experimental methods were similar to those of the curare experiments referred to above. The preparation was irradiated from a small glass tube, 5 mm. long and 1.5 mm. thick, containing emanation, and fixed by means of paraffin to a thin needle, which could be moved towards or away from the preparation by means of the micro-manipulator. The tubes contained 8—50 millicurie of emanation; in order to prevent effects of possible external influences on the preparation it was also covered by a layer of liquid paraffin. These experiments were thus concerned with the effect of the β and the γ rays, especially the former. In order to avoid influencing the measuring instruments themselves the emanation tube was always

kept at the greatest possible distance from them, and careful screening was provided, which proved to be effective in control experiments. The results of these experiments were not quite as constant as those with curare, since irreversible

Fig. 28. Effect of β-rays on end-plate-fibre potentials. 50 millicurie radium emanation 2 mm. distant from the preparation. Grid electrode on end-plate, earthed electrode on the fibre. Electrodes 12—15 μ apart (2 experiments).

Abscissa: time in minutes.

Ordinate: potential in mm. (13 mm. = 1 mV.).

—·——·-·——· end-plate-fibre potential.

— · — · — · — · electrode potential.

- - - · - - - - - - - - · fibre-fibre potential.

↑ application and ↓ removal of the emanation.

(Buchthal & Lindhard).

changes were liable to be produced fairly frequently. The difficulties were, however, not such as to prevent clear-cut experimental results.

As will be seen in Fig. 28 the curve falls, i. e. the *F—E* potential is abolished. After an initial fall for approximately

the first 10 min. which is more abrupt the higher the *F—E* potential, there follows a more slowly declining, almost straight part of the curve, which does not stop at the *F—F* potential but continues until there remains only the potential difference between the electrodes which existed before the experiment. When this stage has been reached the fibre is

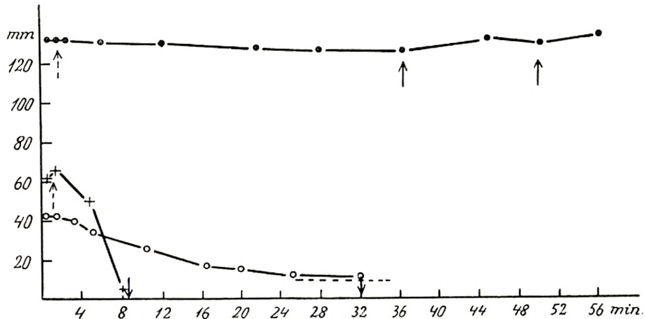

Fig. 29. Potential difference between end-plate and muscle fibre. Upper curve living fibre; Lower curves dying fibres.
↑↑: control of excitability, constant threshold.
↓: preparation unexcitable.
↑: addition of liquid paraffin.
- - - - -: fibre potential.
Ordinate: potential in mm.
Abscissa: time in minutes.
(Buchthal & Lindhard).

no longer excitable directly or indirectly. When the preparation is quite fresh and the experiment has not been of too long duration, the potential differences will reappear when the radium emanation is removed, and the excitability will also return, first the direct and then also the indirect excitability (Fig. 28).

In order to be able to assess these results properly a series of control experiments were made, in order to study the behaviour of the *F—E* potential in fresh preparations, which were covered with paraffin but otherwise undisturbed

apart from occasional testing of the excitability in order to ascertain whether the threshold value was unchanged (Fig. 29, upper curve). Moreover the phenomena seen in dying preparations were examined. In the case of these it appeared, firstly that the *F—E* potential was initially at a comparatively very low level and that as a rule it soon became as low as the electrode potential; secondly that the indirect as well as the direct excitability disappeared simultaneously; and thirdly, that all changes were irreversible in character. At the same time the microscopic picture of the fibre changed, the cross striation first becoming indistinct, then rapidly disappearing. Simultaneously there occur changes in bi-refringence previously mentioned. Finally it was again apparent in the course of the work that direct observation under the microscope is of considerable value in judging the condition of the fibres.

After the experiments reported above, the next step was to examine how the *F—E* potential was influenced by indirect stimulation of the fibre, (Buchthal & Lindhard (1937)). The solution of this problem entailed very considerable changes in the apparatus hitherto employed, as it now became necessary to register potential differences in resting systems as well as changes of rapid and slow character; and above all the possibility of an escape of the stimulus, which has vitiated innumerable experiments in this field, had to be absolutely excluded. These technical difficulties have been satisfactorily overcome by Buchthal & Nielsen (1936).

In order to avoid the possible leading off of current from the muscle cell examined, measurements must be made electrostatically, and since the potential differences that had to be measured were of the order of 10^{-4}—10^{-3} V. very

precise conditions had to be established as regards the input resistance, inertia, sensitivity, and stability of the apparatus. The outcome of this was the construction of a balanced D. C. amplifier with electrostatic input, using the cathode ray oscillograph as recording instrument. The beam of light of the oscillograph was photographed together with the time record on moving film.

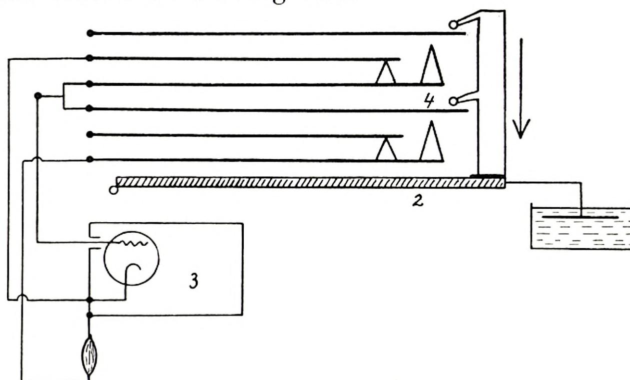

Fig. 30. Contact arrangement (relay) to disconnect and reconnect immediately before and after stimulation to avoid stimulus escape.
1. Glycerine damping arrangement
2. Moved downward by magnet.
3. First stage D. C. amplifier.
4. Contact time varied by insertion of paper here.
(Buchthal).

As the electrical stimuli are the only ones which are quantitatively reproducible this form of stimulation was employed in spite of the great difficulties involved. For if special precautions were not taken the stimulation current would be conducted by the electrodes to the input terminals of the amplifier, where, like the electric responses, it would be amplified and so distort the latter or even obscure them entirely. The difficulties were increased by the fact that the preparation had to be stimulated through the leading-off electrodes, because it was desirable to lead off from the site

of stimulation itself and also because it was inconvenient, for technical reasons, to have three electrodes on the preparation. On the basis of these requirements BUCHTHAL (1937) worked out an arrangement for stimulation which, although the leading-off electrodes were used for stimulating, avoided any spread of the stimulus. This was achieved by interrupting the connection with the measuring instrument during stimulation and re-establishing it within the latent period of the preparation. Thus it became necessary to devise a rapidly working switch system which was able to short-circuit and re-open the amplifier and measuring instruments in about 1 msec. (Fig. 30). Moreover the stimulus had to be a rather high frequency alternating current so as to reduce polarization phenomena at the electrodes.

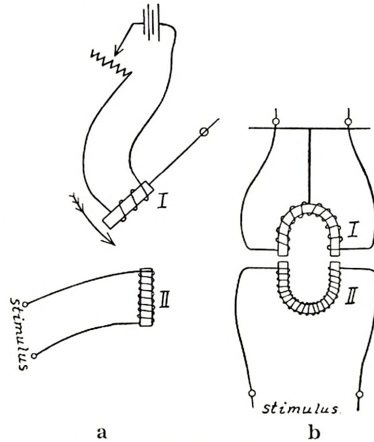

Fig. 31. Device to produce a symmetrical single cycle pulse. a = side view; b = seen from forward.
I = electromagnet attached to the pendulum (300 turns, 0.5 mm. diam. wire). II = stationary coil (1500 turns, 0.1 mm. diam. wire). (BUCHTHAL).

Since the metal switches and electron tube generators employed gave rise to disturbances in several ways BUCHTHAL (1937) introduced a special stimulation device excluding all mechanical contacts (Fig. 31). The stimulus was a single cycle pulse of symmetrical shape (Fig. 32) generated by swinging an electromagnet attached to the Helmholtz-pendulum, past a stationary coil.

BUCHTHAL & LINDHARD's (1937) experiments, using the above methods, gave the following results. In experiments

on single fibres of the frog's semitendinosus a decrease of the resting potential of the fibre was found on direct stimulation, in agreement with Buchthal & Péterfi's results (1934), the resting potential being re-established in a period of restitution that lasted for a considerably longer period than the mechanical response. On direct stimulation when one electrode had been placed near the nerve ending, indicated by the fact that the intensity of the stimulus could be lowered considerably, two different changes in the potential occurred successively and could interfere to some extent. The first rapid potential change (the *F—E* potential) appeared 1—2 msec. after the excitation and lasted for 2—3 msec. Then followed the change in contraction potential, sometimes after a short isoelectric interval. The direction of the two potential changes relative to each other was, of course, responsible for the shape of the records. Fig. 33 represents one curve, in which the two potential changes are separated by an isoelectric interval and both go in the same direction. Both potential changes are diminutions of the *F—E* and *F—F* potentials respectively. After the experiment the fibre gradually became inexcitable, even when strong stimuli were employed; the resting potential of the fibre had then disappeared. Then a potential difference was produced between the electrodes from a voltage source in series with the earthed electrode and the dead fibre was then stimulated. Fig. 33 a shows that in these records of controls the voltage of the non-reacting fibre is completely unaffected by stimulus escape. The downward spike (shown by arrow in Fig. 33) in this,

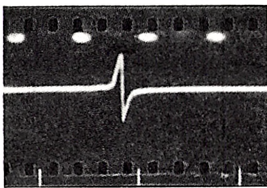

Fig. 32. Record of the single cycle pulse (cathode ray oscillograph) produced by the device shown in Fig. 31. Time in 1/50 sec. (Buchthal).

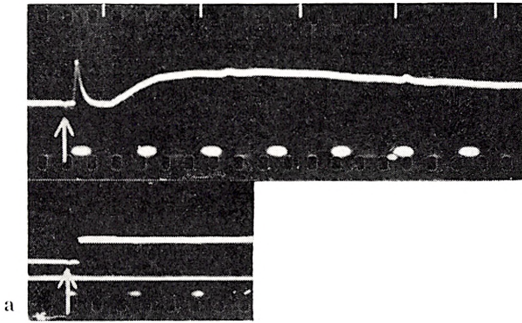

Fig. 33. Potential difference accompanying excitation and contraction of an indirectly stimulated frog's muscle fibre. Electrodes 300 μ apart. Potential (4 mV.) of the resting fibre compensated. Upward movement, grid electrode more negative. The spike over the arrow marks moment of stimulation. Distance between two light marks 20 msek. a. Control for stimulus escape by stimulation of unexcitable fibre after introducing a direct current of 0.5 mV. via the electrodes. (BUCHTHAL & LINDHARD).

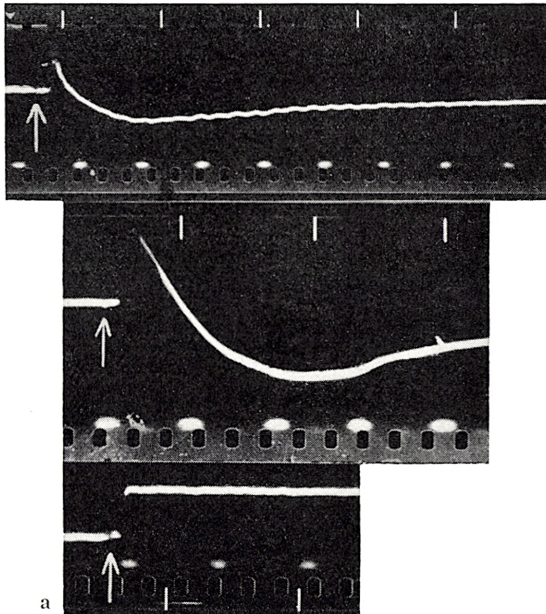

Fig. 34. Potential difference accompanying excitation and contraction. Indirectly stimulated frog's muscle fibre. Excitation and contraction potentials vary in opposite directions. Electrodes 300 μ apart. Compensated potential of the resting fibre 3.5 mV. For (a) see Fig. 33 a. Time marker 20 msek. (BUCHTHAL & LINDHARD).

as well as in all other curves indicates the moment of stimulation and not the moment of connection with the amplifier, which occurs a little later. Fig. 34 reproduces an experiment in which the two potential differences, in this case moving in opposite directions, interfere to some extent. The resting

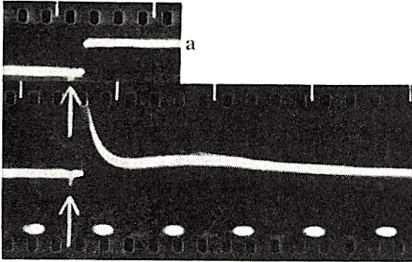

Fig. 35. Potential difference accompanying stimulation and contraction of a lizard's fibre. Grid electrode on the motor end-plate. *E*—*F*. potential compensated 35 mV. For (a) see Fig. 33 a. (Buchthal & Lindhard).

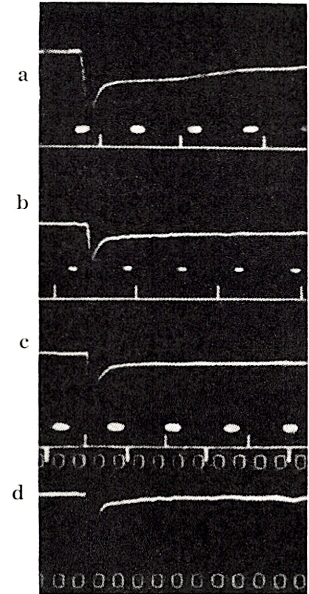

Fig. 36.

Fig. 36. Lizard's fibre. Influence of different electrode separations. One electrode remains on the end-plate. *F*—*E*. Potential 35 mV., compensated. (a) distance 400 μ. (b) distance 250 μ. (c) distance 150 μ. (d) distance 50 μ. Time marker 20 msec. (Buchthal & Lindhard).

potential in all experiments was compensated, only the changes in potential differences being registered.

It was thus possible, in the case of frog muscle fibres to distinguish between changes in excitation potential (*F*—*E* potential) and in contraction potential. Closer examination of the former made it necessary to carry out experiments on lizard's muscles, in which it is possible to lead off from and to stimulate directly the motor end-plate. The potential difference between fibre and end-plate, the *F*—*E* potential, previously referred to, was compensated so that only the

alterations of potential were registered. About 1—2 msec. after stimulation a fall in the *F—E* potential occurred, amounting to about $^1/_{10}$ of its resting value, after which in the course of 1—2 msec. the potential regained its former value, the latter part of the curve interfering with the oscillations of the *F—F* potential. Fig. 35 shows the appearance when the two potentials change in the same direction. Fig. 35 a represents a control curve on an unexcitable fibre obtained as above. In this case, too, the potential change has been reproduced without any disturbance whatever. The relation between the variations of the *F—E* and the *F—F* potentials will appear more clearly when the distance between the electrodes is varied, though the distance available is not great enough to decide whether the potential change is propagated or spread with decrement.

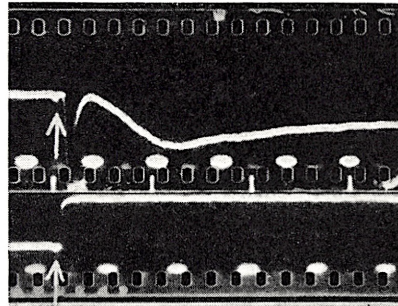

Fig. 37. Lizard's muscle fibre, indirectly stimulated by eddy currents, both electrodes on the muscle fibre. *F—F.* potential 2 mV. compensated. Electrodes 400 μ apart. For (a) see Fig. 33 a. Time marker 20 msec. (BUCHTHAL & LINDHARD).

The grid electrode is placed on the end-plate whilst the earthed electrode is placed on the fibre at varying distances from the end-plate, the resting potential being compensated as previously. The curves in Fig. 36 show how the potential difference varies under these conditions. If both electrodes are placed on the fibre, and one of them close to the end-plate, the latter thus being stimulated by eddy currents, a curve like that in Fig. 37 will appear, i. e. an excitation potential followed by a contraction potential corresponding in all respects to the one found in the frog's muscle fibre.

In order to secure direct stimulation of the fibre a number of experiments were performed on curarized lizard's fibres. The grid electrode was placed on an end-plate as usual, the earthed electrode on the fibre. The curare solution was applied to the preparation and it was found as previously that the *F—E* potential gradually diminished simultaneously with an increase in the threshold value of the stimulus. After 10—30 minutes, only the *F—F* potential remained, the end-plate was electrically inactive. Direct stimulation is then not accompanied by two separate changes but by a single abrupt decrease of the fibre's resting potential. The more abrupt initial fall found here is probably due to a greater density of current under the stimulating electrode. Such experiments must be very carefully controlled, because the intensity of the stimulus is necessarily many times greater than in the case of indirect stimulation. The potential changes of a curarized fibre are reproduced in Fig. 38. As usual in these experiments the resting potential was compensated, only the abrupt decrease and slow regeneration of the potential being seen in the curve.

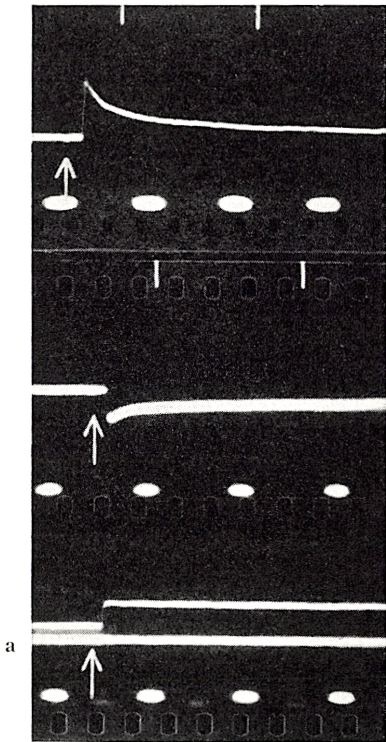

Fig. 38. Variation of potential difference on stimulation of completely curarized fibres. Fibre potential, 3 mV. compensated. Electrodes 200 μ apart. For (a) see Fig. 33 a. Time marker 20 msec. (Buchthal & Lindhard).

In certain cases the *F—E* potential is found to have disappeared after 10—15 minutes, whilst the indirect excitability remains for a further 15 minutes or so. Since the potentials are led off through the sarcolemma, it is reasonable to suppose that when a potential can no longer be detected by the recording instruments, traces of the latter will still be found in the fibre. As is well known, it is possible to stimulate a single fibre without including neighbouring fibres in the process, the interpretation of which seems to be that the sarcolemma is a comparatively effective insulator. These experiments show once again what care must be taken in order to secure complete curarization of a muscle fibre. Where experimental conditions must be exactly controlled, curarization of whole muscles is a hopeless undertaking. It may be that the results published by SCHÄFER (1936) and ROSEMANN (1936), which differ from ours, are partly explained by that fact. Moreover, our experiments with curare show that the very rapid change of the *F—E* potential which takes place first of all, does have its origin within the fibre end-plate system, and thus is not an action current of nervous origin; for curare would not affect the latter.

This series of experiments confirms the opinion previously expressed, that the problems with which we are dealing, like several others in muscle and nerve physiology, can only be solved by the use of the functional units themselves. The complicated conditions presented by the whole muscle, composed as it is of fibres, end-plates, nerve twigs, blood vessels, connective tissue and tissue fluid, makes conflicting results almost inevitable.

Direct measurements on end-plates and fibres, on the other hand, give uniform results; it shows that between

these structures there is a difference in potential, which on indirect stimulation undergoes a sudden change, spreading over the fibre as a wave[1], and whose relation to the fibre potential has been recorded. The motor end-plate is morphologically and functionally a highly specialised region of the fibre, as LINDHARD has pointed out (1924, 1931); and it appears probable that KEITH LUCAS' β-substance, which, as is well known, is characterized by high excitability, might be referred to this region. Some of the electrical changes taking place on stimulation of the fibre are thus due to changes in the F—E potential, as could be inferred from ASMUSSEN & LINDHARD's experiments already referred to; but the changes in the F—F potential could not be demonstrated with the experimental arrangement they employed. Furthermore, BISHOP & GELFAN (1932) did not succeed in demonstrating electrical changes when localized contractions were produced in muscle fibres by direct stimulation. The quantitative determination of electrical changes occurring in the single fibre, and especially their time relations, only become possible when electrostatic recording is employed and the measuring device has an input resistance of at least 10 meg. ohms; if this is not the case the fall of voltage in the preparation may result in considerable errors. The experimental results reported here are not yet applicable to whole muscles; but by employing the electrostatic methods of measurement on whole muscles we can, in certain cases, obtain results which correspond qualitatively with those arrived at in experiments on single fibres. (Fig. 39.) In particular, it appears that the electric phenomena in muscle are associated with the process of excitation as well as that of contraction.

[1] Not to be confused with the so-called contraction wave observed in whole muscles.

Although the former experiments on whole muscles, for reasons previously discussed, do not permit definite conclusions to be drawn concerning the electrical changes in the muscle substance, the experiments described above on single fibres have shown beyond all doubt that besides the potential changes in the transmitting system end-plate-fibre, potential changes also occur in the muscle substance proper.

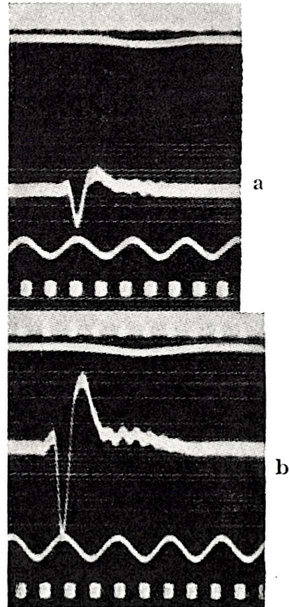

Fig. 39. Mechanogram and action current of M. semitendinosus (frog) stimulated over the nerve. (a) input resistance of the electrone tube voltmeter = 3000 Ω. (b) = 40 Meg. Ω. Time 1/50 sec. (Buchthal & Lindhard).

In the question as to the precise mechanism of the transmission of impulses to striated muscle, the rôles of acetylcholine on the one hand, and the electrical phenomena on the other, have been the subject of much discussion (Brown, Dale & Feldberg (1936), Monnier (1936) Dale (1937)).

The transmission of excitation from the nerve-ending in the end-plate to the sole of that organ need not necessarily be of the same nature as the process taking place between end-plate and fibre-substance. It seems advisable in future work to differentiate between these two boundaries. It may be mentioned that experiments in which acetylcholine was applied directly to single end-plates, indicate that the effect of this substance is more complicated than has been hitherto supposed. The process of excitation, as it is usually understood, involves several stages, in which transmission by both physical and chemical means may be involved.

The Spread of the Stimulus.

As already mentioned, the older conception was that the process of stimulation spread as a "wave of negativity", which advanced from the point where the nerve entered the whole muscle at the rate of the wave of contraction. On anatomical grounds this theory is untenable and it cannot be made the basis for further work. A more productive idea was Keith Lucas' (1905) that each fibre of a skeletal muscle reacts according to the so-called "all-or-none" principle, which for a long time had been considered to apply only to the myocardium. Keith Lucas showed that a frog's muscle, when excited by stimuli of gradually increasing strength, responded with a "stepped" contraction curve; the author's explanation was that with increasing intensity the stimulus spread more widely in the muscle and thus would successively attack one "set" of fibres after the other. And as the "steps" of the stepped curve were horizontal, this was interpreted as a maximal reaction of each new set of fibres. The latter conclusion was not entirely justified, however, as the production of a stepped curve does not necessarily result from the maximal response of the fibres, but might equally well have its origin in a constant, and not necessarily maximal, reaction of each. This might be the case, even though an increasing stimulus is applied to the muscle as a whole.

Some uncertainty existed as to the precise condition of stimulation in these experiments of Keith Lucas and his school; both he and later workers found stepped curves both on direct and indirect stimulation and on fresh and curarized muscles. Therefore it was considered necessary to define more precisely the relation between direct and

indirect stimulations as well as to examine closely the behaviour of curarized fibres.

The question of direct and indirect stimulation has been dealt with in a number of classical experiments by the German physiologist SACHS (1874) who examined the effect of stimulation of a muscle in the longitudinal and transverse directions of the fibres. These experiments have now been extended, and their results confirmed, by ASMUSSEN (1933). The procedure was as follows:— Two Gastrocnemii of the same frog were suspended in the same myograph under identical mechanical conditions and their contractions registered simultaneously. The muscles were stimulated directly and indirectly, and in the former case either along or across the direction of the fibres. It then appeared that by indirect or direct stimulation along the fibres, almost identical "stepped" curves were obtained, though with indirect stimulation the steps were somewhat more pronounced and shortening slightly less (Fig. 40). In other words, the so-called "direct" stimulation along a non-denervated muscle is chiefly of an indirect nature; it is mainly the intramuscular nerve branches which are stimulated, as HAPPEL (1926) had already shown.

In certain cases an underlying effect, due to true direct stimulation, may result in a moderate increase in shortening. If, on the other hand, the muscle is stimulated transversely it is found that firstly the strength of the stimulus must be increased very considerably before the muscle will react at all, and secondly that the curve as a rule shows no signs of "steps" (Fig. 41). In other words, in this case stimulation is mainly direct. However, by increasing the strength of the stimulus very slowly, fairly well-marked "steps" in the contraction curve produced by transverse stimulation

may sometimes be found. These are presumably due to direct effects on the motor end-plates. With gradual increase of the stimulus a perfectly smooth rising curve is obtained only when the muscle is "denervated" in some way; and, for this reason, the majority of the experiments in which "direct" stimulation of non-curarized muscles was employed must be considered of doubtful value. But this does not

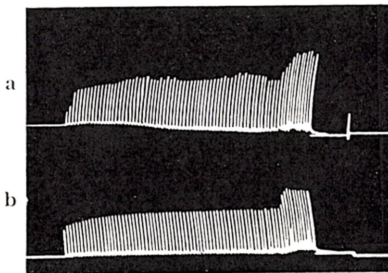

Fig. 40. Frog's muscle. (a) "direct longitudinal" stimulation. (b) indirect stimulation. (Asmussen).

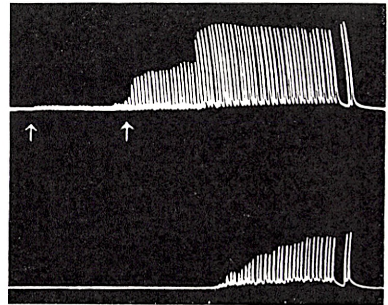

Fig. 41. Upper curve: indirect stimulation; between the arrows a variable resistance was gradually removed. Lower curve: direct transverse stimulation. (Asmussen).

imply that curarized muscles can always be used in these experiments; it has already been mentioned that a great deal of work reported in the literature can be said with certainty to suffer from the serious defect that curarization has been incomplete.

It is obvious that experiments using mixed direct and indirect stimulation on muscles only partially curarized are unsuitable for the study of the problems discussed here. Despite several notable advances in technique, e. g. Pratt & Eisenberger's pore electrodes, some confusion still existed, mainly owing to the use by many workers of unsuitable preparations (the basihyoid membrane) as at that time it had not been found possible to use isolated fibres.

Brown & Sichel first succeeded in doing so, and shortly afterwards Asmussen and Kato. Brown & Sichel (1930, 1936) showed that a muscle fibre stimulated directly did not follow the "all-or-none" law but responded with contractions which increased with the intensity of the stimulus. This was in agreement with the work of Gelfan (1930). Asmussen (1931, 1932), who undertook a systematic study of the mechanical properties of the isolated fibre, chiefly employed Brown and Sichel's technique, though he also made use of small bundles, to some extent. The procedure is illustrated in Fig. 42. As will be seen, the arrangement does not record completely isometrically, but the changes in length become so small in proportion to the fibre length that the curves obtained may be considered as isometric tension curves. The result of Asmussen's work was as follows:— With direct stimulation of constant strength the tension was constant also, but sooner or later, a "contraction-remainder" developed, i. e. relaxation gradually passed off more and more slowly so that after each contraction the fibre did not return to its initial length or tension. Thus it appeared that the increase of tension was constant with each successive stimulus (Fig. 43). In other words it was not the tension but the increase of tension that varied with the strength of the stimulus. After cessation of the stimulus the tension disappeared. With the onset of fatigue, both the increase in tension and the "contraction-remainder" steadily approach zero (Fig. 44).

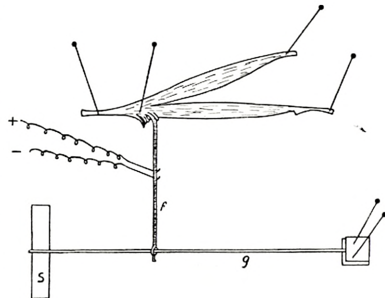

Fig. 42. Arrangement to record tension of a single fibre. Frog's M. semitendinosus. f = single fibre with electrodes. g = glass rod. s = scale. (Asmussen).

If the strength of the stimulus varies, it appears, in accordance with Brown & Sichel's results, that the tension

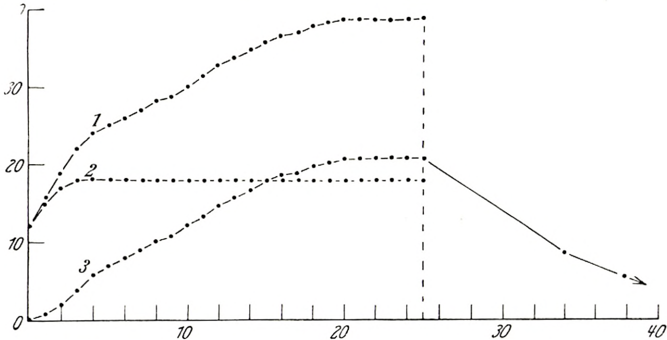

Fig. 43. Single muscle fibre, direct stimulation with constant strength and frequency. 1. Total tension. 2. Increase of tension for each stimulus. 3. Change of base line.
Abscissa: time in 0.8 sec. Ordinate: arbitrary units.
(Asmussen).

will vary accordingly. If the intensity of the stimulus is kept constant whilst its frequency changes, the response of the muscle with increasing rate of stimulation, will gradually pass from a series of single contractions into an almost

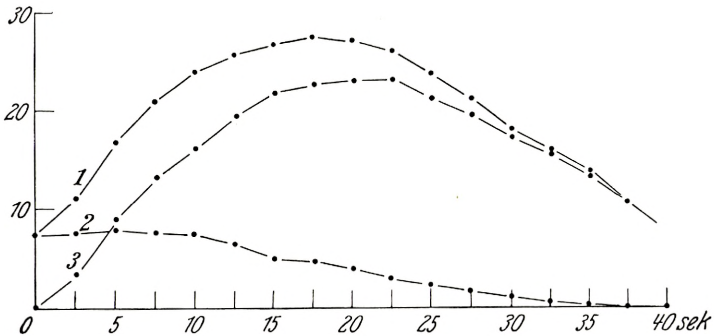

Fig. 44. Single fibre, direct stimulation continued until onset of fatigue. For 1, 2 and 3 see Fig. 43.

smooth curve of interference, the so-called tetanic curve. The latter will, however, not run parallel with the base

line but will be a rising curve. Since this curve represents the total tension of the fibre it will be obvious, from the above, that this total tension, denoted by the "height of contraction" will depend upon the frequency of stimulation. The more rapid the rhythm, the higher the total tension; for the greater will the "contraction-remainder" become in a given time. If the intensity of the tetanizing stimulus is increased, the curve of tension will rise correspondingly.

These experiments show that on direct stimulation the isolated fibre does not follow the "all-or-none" law.

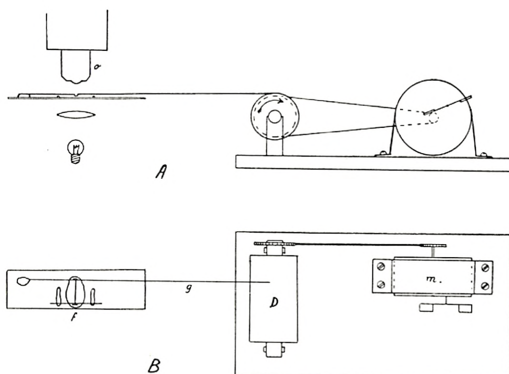

Fig. 45. Device for recording tension of single fibres. A. side view. B. seen from above. f = fibre. g = glass rod. D = drum driven by clockwork motor. o = microscope objective. (Asmussen).

In experiments with indirect stimulation Asmussen (1934) employed a somewhat modified experimental technique (Fig. 45). The preparation employed was the same as in the experiments first referred to, namely isolated fibres or small bundles of the frog's Semitendinosus. In some experiments lizard's muscles were employed.

The experiments showed that despite large increases in the strength of the stimulus, the contraction and the increase of tension of the isolated fibre remained constant (Fig. 46 a). However, on excitation of a bundle with increasing stimulus, a stepped curve was obtained which possessed as many steps as the bundle contained fibres. Kato and his collaborators have obtained similar results. If stimulation is

continued at constant strength the bundle will become fatigued, and a curve of tension falling stepwise will then be

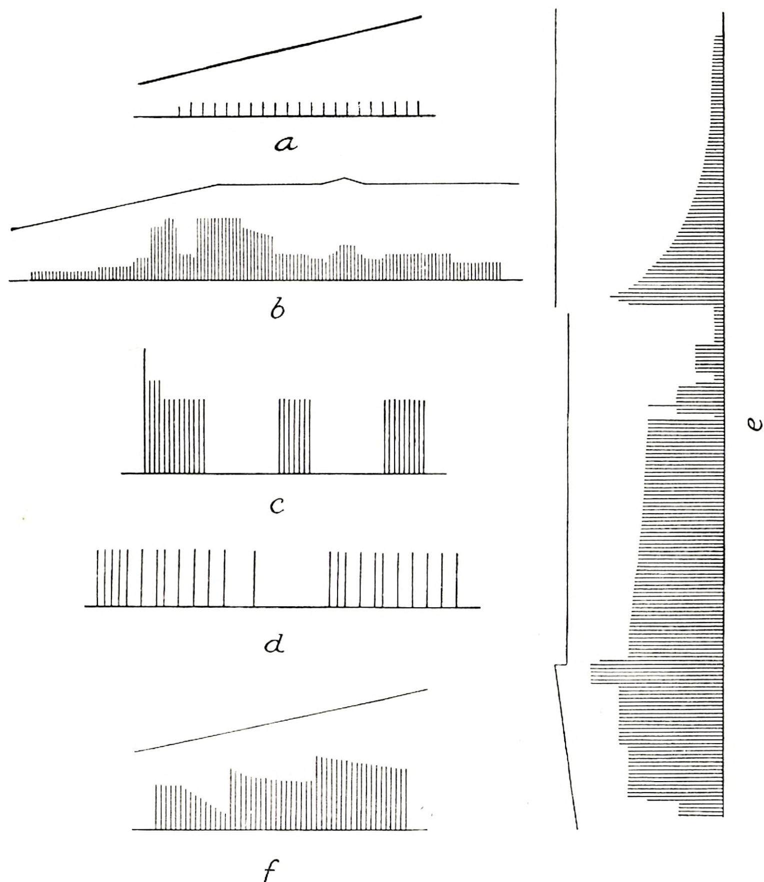

Fig. 46. a. single fibre (lizard); indirect stimulation; upper curve indicates strength of stimulus. b. small bundle of 8 fibres, indirectly stimulated; upper curve strength of stimulus. c. small bundle, continuous indirectly stimulated, strength of stimulus constant (compare Fig. 47). d. small bundle indirectly stimulated with constant frequency, threshold stimulus. e. response of muscle bundle. Upper curve strength of stimulus; left side indirect, right side direct stimulation. f. response of muscle bundle, indirect stimulation. Upper curve strength of stimulus. (Asmussen).

obtained. These stepped curves are not due to fatigue of the peripheral nerve, since as is well known this is an extremely

slow process; they are moreover not due to fatigue of the fibres themselves, since these will still respond to direct stimulation, and with the onset of fatigue show a smoothly falling curve. ASMUSSEN's explanation is that they are due to an "all-or-none" reaction of the end-plate. Fig. 46b shows how "end-plate fatigue" affects the response of the bundle. It is seen from Fig. 46 a—d that even if the tension has fallen to zero the fibre will, after some time, respond afresh to the same or a slightly increased stimulus, and then again cease to react. If the fibre has become greatly fatigued by indirect stimulation, and a change is then rapidly made to direct stimulation, the strength of the stimulus being increased simultaneously, a very powerful response may be obtained for a short time, which soon passes into a continuously declining curve of fatigue (Fig. 46e). Thus it is possible from the shape of the curve of fatigue to decide whether the site of the fatigue is the end-plate or the contractile substance.

In some cases (Fig. 46e) the two forms may be combined. The intermittent fatigue curves show that on indirect stimulation the fibre does not fatigue in the same way as on direct stimulation. If the curve of contraction in the case of direct stimulation falls to zero, the fibre has reached the stage of absolute fatigue and cannot be made to respond again by increasing the stimulus; only after a comparatively long rest will it be possible to make it react again. In the case of an intermittent fatigue curve there are, however, two possibilities. Either the motor end-plate may become fatigued, as ASMUSSEN suggests, or the excitability of the muscle fibre suddenly may decrease so much as to make the constant stimulus initiated from the end-plate "sub-threshold". It is quite impossible to decide with certainty

which of these alternatives is realized under normal conditions; but in certain cases the muscle may be fatigued without the intervention of the end-plate, so that the muscle tension decreases towards zero whilst the impulses from indirect stimulation indicated by action-currents continue with unaltered intensity (HENRIQUES & LINDHARD). This is probably the explanation of Fig. 46f; here fatigue of the muscle fibre is manifest before any signs of this have appeared in the end-plate. An experiment such as that reproduced in Fig. 46d, where contractions recommence as the result of an increase in indirect stimulation would appear however to indicate that in this case we might really speak of fatigue of the end-plate. Considering the very short refractory period of the end-plate, it seems likely that when the fibre ceases to act, this is due to a decreased excitability owing to fatigue (cf. e. g. Fig. 47). As previously mentioned, however, we have in all cases to reckon with the fact that the normal stimulus, as indicated by the change in the *F—E* potential, is constant under given experimental conditions within rather narrow limits. The next question will then be whether this constant stimulus is maximal as regards the fibre. KEITH LUCAS believed this to be so, because the steps of the stepped curve were horizontal. This fact, however, as already mentioned, proves only that the stimulus is a constant one and that the fibre is in good condition. In order to investigate this question more closely ASMUSSEN & LINDHARD (1935) compared the maximal contraction of the whole muscle and the single fibre with direct and indirect stimulation.

Fig. 48 shows the behaviour of a frog's gastrocnemius under a tetanus of short duration, produced by a very strong stimulus applied directly, and also to the motor nerve.

Though the direct stimulus might not be maximal since the arrangement employed did not permit further increase in the strength, it is nevertheless certain that the indirect

a
b

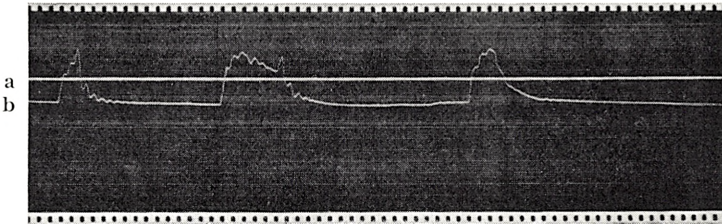

Fig. 47. Tension of a whole muscle (a) and of one of its fibres (b); indirect stimulation (compare Fig. 46 (c). (ASMUSSEN)).

stimulus employed is a maximal one, in so far as there would be no purpose in increasing it. It will be seen that the muscle tension falls rapidly, as would be expected, but further that in every case it is greater in direct than in indirect stimulation. The same result is shown in Fig. 49,

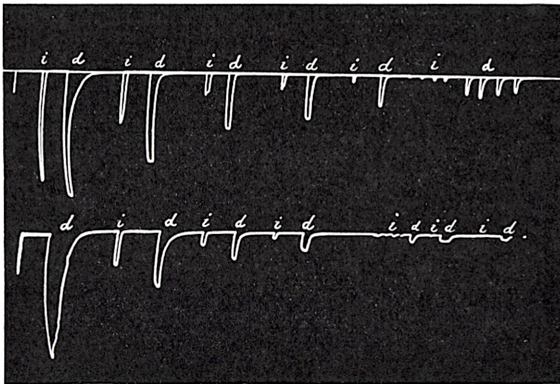

Fig. 48. Frog's muscle; indirect (i) and direct (d) stimulation. (ASMUSSEN & LINDHARD).

an experiment with an isolated fibre; the maximal tension is far greater in the case of direct than with indirect stimulation. This must mean that an indirect stimulus is not maximal

as regards the response of the fibre so that the expression "all-or-none" cannot be used at all in the case of a fibre of a skeletal muscle, but that we really have to do with a constant reaction. Nor can we speak of the all-or-none law in the case of the *F—E* potential. This term, therefore, should be discarded, the more so since it probably does not apply to the myocardium itself either but only to the excitation of this (Schütz, 1936).

As to the spread of the stimulus in the muscle we constantly find the term "wave of contraction" in the older literature. This wave was imagined as a gross alteration in shape, which could be recorded mechanically, especially when alterations of thickness were measured. This "wave of contraction" must be dismissed from consideration, at any rate when normal function of the whole muscle is concerned. It is true that, from a consideration of the experiments of Fuji and others, we may suppose that the latent period of the different fibres varies, but that the individual fibres are not so situated as to divide the muscle into separate regions, which can contract individually; on the contrary, they are situated as far as is known, so that fibres innervated from the same ganglion cell are lying scattered in the muscle and therefore a partial muscular contraction will appear as a contraction of the whole muscle of decreased amplitude. As regards the individual fibre, hundreds of microphotographs taken by Buchthal, Knappeis & Lindhard at, and immediately after, the moment of stimulation in no case give any indication that the muscle

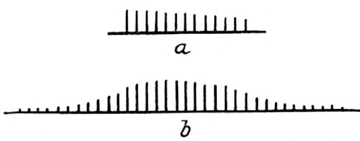

Fig. 49. a. single fibre, indirectly stimulated; commencement of fatigue. b. direct stimulation of the same fibre; strength of stimulus first increasing then decreasing. (Asmussen & Lindhard).

compartments in the field of the microscope are in different states. In this connection it may be borne in mind that from the standpoint of energetics the older conception of the contraction wave in the whole muscle would be futile as it would result in a series of useless tensions in the fibres, which during the whole of the process of contraction would be prevented from attaining a state of equilibrium.

ASMUSSEN & LINDHARD (1935) have examined the spread of the stimulus in a fibre or a small bundle by means of a modification of a method first devised by KATO (1934) and reproduced in Fig. 50. The preparation, covered by a very small quantity of Ringer's solution, is fixed to a slide in its centre with a little plaster of Paris; then to each end is attached a long thin glass-rod writing on a drum. The preparation is stimulated at one end by condenser discharges through platinum electrodes, or indirectly through its nerve branch. If the preparation was completely symmetrical and attached completely symmetrically, the glass-rods of equal thickness and the amount of their friction on the drum quite uniform, and lastly, the muscle fibre attached to the fulcrum at the same distance from the ends of the two glass-rods, then two symmetrical curves should be obtained on the drum, if the stimulation passed through the whole of the fibre. Owing to the numerous sources of error, which will not be dealt with in detail here, such symmetrical curves are rarely obtained, but on the other hand when these

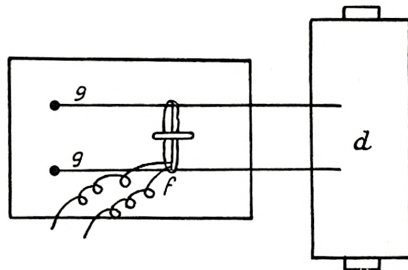

Fig. 50. Recording device for registering tension at both ends of the same fibre. gg = glass rods, d = recording drum, f = fibre fixed in the middle by plaster of Paris. Electrodes attached to the one end of the fibre.

sources of error are taken into account asymmetry will not become so great as not to allow certain conclusions to be drawn from the experiments as to the spread of the excitation in the fibre.

On indirect stimulation of a bundle the symmetry is so marked that it cannot be doubted that the spread of the stimulation is rapid and decrementless. It was necessary

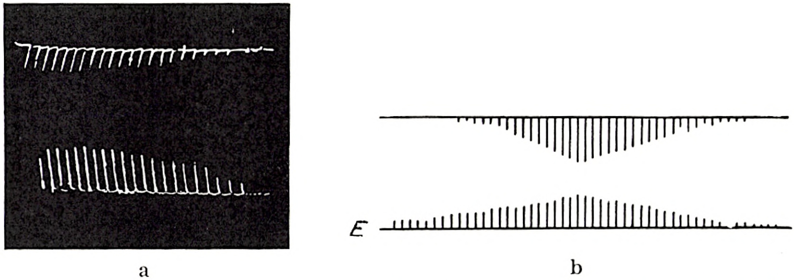

Fig. 51. a. Tension record from both ends of a fibre fixed at its middle. Direct stimulation of increasing strength. E denotes record from portion of fibre on which electrodes are placed. b. As (a); strength of stimulus first increasing then decreasing. a = original record, b = drawing from record. (Asmussen & Lindhard).

to assume beforehand that as stimulation from the end-plate is constant any portion of the fibre that remained uninfluenced by the stimulus would be unable to contract at all under normal conditions.

On direct stimulation some variation in the appearance of the curves is found owing to the uncertainty associated with the method employed. There seems to be a tendency for visible contractions to begin at the stimulated end of the fibre or the bundle, but this tendency is far from being so marked that it would be possible to express it numerically (Fig. 51 a & b). For further details the original paper should be consulted. It appears with certainty from the experiments that the stimulus passes throughout the fibre and that the

contraction thus becomes complete at an intensity below that of the normal stimulus which may therefore be regarded as being above the threshold. Although it does not appear certain from these experiments that a fibre can be stimulated partially, the observations nevertheless show that the contraction becomes complete a long time before it becomes maximal. By other means, however, especially by stimulation of single fibres with microelectrodes (BUCHTHAL & PÉTERFI) (GELFAN) or mechanical stimulation (ASMUSSEN & LINDHARD) under the microscope, it has been demonstrated by direct observation that in the case of weak stimulation a localised contraction may appear at the site of stimulation.

For many years a major problem of muscle physiology was the so-called "latent period" i. e., the period between stimulation of the motor nerve and the mechanical response of the muscle as measured by the curve of a single twitch. So long as we regard the whole muscle as a single unit such a viewpoint is natural; but gradually it has come to be generally realized that all curves obtained in such experiments are interference curves, often very difficult to interpret quantitatively; and for this reason, the question of the latent period of the whole muscle has lost in interest. At the beginning of this century it was realized that something took place during the latent period, fibrillary twitching of the muscle belly was observed and it was seen that the lever of the myograph fell below the base-line during the latent period indicating altered conditions of elasticity. It was observed that with improvement in the apparatus used the latent period constantly decreased. A new attempt was made in 1925 by FULTON to analyse the phenomenon; it proved unsuccessful, however, because it was not appreciated that all such curves are interference phenomena and

because the structure situated between nerve and muscle viz. the motor end-plate, was not taken into consideration. The curve of the single twitch, too, is but an inadequate statistical expression of what happens in the muscle after stimulation (cf. Fuji's experiments). This is shown, in a somewhat exaggerated form perhaps, by Fig. 52.

The impulse initiated in the nerve by the stimulus reaches the different fibres by routes of unequal length, causing the fibres to contract at slightly different moments, so that a considerable number of fibres must be set in action before the lever can be moved. The latent period of the muscle measured from this curve will thus be the period between stimulation of the nerve and the contraction of a certain unknown number of fibres. Then we must consider the "height of contraction", from which it is supposed to be possible to calculate the shortening of the muscle. In this case at any rate it cannot be done even with moderate accuracy; because owing to its kinetic energy the heavy lever must have risen further than really corresponded to the shortening of the muscle; when a sufficiently large number of fibres have relaxed it falls down to, and below, the baseline again. Even if it was really possible to learn something about the shortening of the muscle, we should learn nothing about the far more interesting phenomenon, the shortening of the fibre, as this rarely takes place in the direction of the pull of the whole muscle.

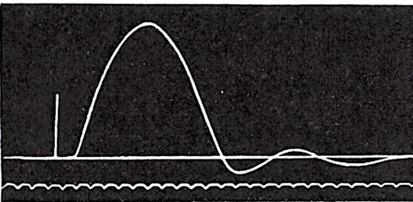

Fig. 52. Single twitch of whole muscle. (Beddard).

It will probably appear from the above that the old idea of the latent period has been played out. The process

of stimulation is a complex physico-chemical process, and at each stage of the various parts of this we may, of course, speak of a latent period, but this will be quite another thing than that previously understood by the term "latent period", something for the measurement of which quite different methods are necessary, and first and foremost, requires the use of isolated fibres. A few experiments of this nature have already been made, as BROWN & SICHEL (1936) have determined the time interval between the stimulus and the beginning of the mechanical response, which they state to be 1.5—2.5 mill. sec., whilst BUCHTHAL & LINDHARD have determined the time interval between the stimulus and the fall in the *F—E* potential, which amounts to 1—1.5 mill. sec., and the interval between the fall of the *F—E* and the *F—F* potentials, which is of the same order.

It has been mentioned several times that frequent stimulation of a fibre may bring it into a state of permanent contraction which, to use an expression borrowed from experiments with whole muscles, has been termed "tetanic contraction" or "tetanus". As already referred to, this condition is, however, labile and can only be maintained for a few seconds at the most. EISENBERGER (1918) states that sometimes it is impossible to keep a fibre tetanized long enough to photograph it in this condition, which may perhaps be due to the fact that he uses fibres in situ and not isolated, which would entail a fairly strong tension on the single, contracted fibre, and therefore presumably, fatigue of earlier onset. Here again there is a contrast between observations made on single fibres and on whole muscles, which is scarcely to be wondered at. As we have said, the so-called single contraction curve is formed through interference of

the curves of the individual fibres; the tetanic curve arises through summation of such interference curves. Therefore it is almost hopeless to attempt to analyse a tetanic curve, whether it be in mechanical or thermal problems. It is unfortunate for the progress of muscle physiology that this highly complicated phenomenon has been characterized as a special "form of contraction".

As is known, a muscle may remain in "tetanic contraction" for several minutes, provided the stimulus is of suitable intensity. When maximal stimuli are employed the curve, after rapidly reaching a maximum, immediately falls to the base-line. It might, therefore, be imagined that it was for similar reasons that the isolated fibre can only remain in tetanic contraction for a brief space of time, since the intensity of the natural stimulus lies far above the threshold value, as has already been mentioned. This fact is, however, undoubtedly only of minor importance here. The main reason why a muscle can remain in tetanic contraction for minutes at a time is that single fibres or groups of fibres alternate during the contraction. That this does actually occur has been shown by ASMUSSEN (1934) in the following way. He dissected out a single fibre so that it was connected only by its nerve with the remaining muscle, both being arranged for isometric contractions. When stimulated by faradic current the whole muscle gave a perfectly smooth tetanic curve, parallel to the base-line. The single fibre, on the other hand, gave an intermittent curve consisting of a series of tetani of short duration (Fig. 47). These experiments are in agreement with those above on isolated fibres and explain why the tetanus is of longer duration in the whole muscle. The single fibre, either because its end-plate becomes fatigued or, what is more probable, because its

excitability is diminished, becomes irresponsive to stimulation for a short period, contracting again after this pause. The experiments also explain why in the case of maximal stimulation the muscle cannot remain long in tetanic contraction; the maximal stimulus acts on all the fibres simultaneously and, therefore, they will very soon all cease to act; there will be no possibility of alternation between different groups of fibres.

It is difficult to ascertain from the literature whether it has been realized that all these so-called contraction forms should properly be termed innervation forms, since the underlying process in the fibre or, more precisely, in the muscle compartment is the same, qualitatively, and in certain cases (indirect stimulation) quantitatively also. A muscle which, indirectly stimulated, can without alteration in length support a given weight, will if the strength of the stimulus is increased, raise the weight and so perform an amount of external work (concentric contraction); if the strength of stimulus diminishes the weight will be lowered and the muscle will perform a negative amount of work (excentric contraction). A loaded fibre stimulated indirectly will be able to contract only for a very short period; but it will not be able to raise or lower the load because the indirectly stimulated fibre has only one degree of contraction. Only if the load can be varied will the fibre be able to perform a positive as well as a negative amount of work. But the directly stimulated fibre, like the whole muscle, has an "infinite" number of degrees of contraction.

It was a long time before a contraction of normal type could be obtained from a muscle dissociated from the central nervous system. When at last it became possible (Grace Briscoe, 1927) it was not because a closer knowledge

of the nature of muscle contraction had been attained, but because it was realized that the question involved was one of innervation.

The Reaction of the Muscle Fibre to the Stimulus.

Although there exists an enormous literature on the reactions of whole muscles, only in a few cases have single fibres been studied. The results of experimental studies on the reactions of whole muscles are even more difficult to interpret than those previously referred to. The painstaking work of the past three decades on muscle chemistry, aiming at establishing a direct relation between chemical and thermal changes, and the mechanical phenomena, has proved disappointing for several reasons. This is due partly to the fact that the chemical changes cannot be limited as to time so as to make it possible to follow directly the different links of the complicated biological processes taking place in rapid succession in the muscle fibre, and moreover we cannot be certain whether the substances found in the dead fibres exist as such in the living state; lastly, the determinations have been performed on a mixture of substances from all the structural elements present, not only in the fibre but in the whole muscle. Therefore, recent investigations of the physico-chemical basis of the contraction mechanism have followed quite other lines than previously, which have been referred to briefly in the chapter on the minute structure of the muscle fibre.

As far as the thermal investigations are concerned, the problem is even more difficult, because the heat change that is measured, and which attemps are made to relate to the known chemical changes, may have no immediate

quantitative connection with the latter. It is true that a successful distinction may be made between contraction heat and recovery heat (of chemical origin), but the highly complex conditions existing in intact muscles during tetanus where we are concerned with the summation of interference curves, do not allow us to relate the various heat changes observed to definite processes in the single fibre; we know nothing at all as to the number of reacting fibres, though we do know that the fibres reacting to a given stimulus do not do so simultaneously. Complications therefore arise when attempts are made to refer the heat changes quantitatively to the mechanical processes. Apart from the fact that the mechanical phenomena dealt with in these experiments can only in part be considered as immediately associated with the process of excitation, there are several facts to be discussed below which will influence the results of the experiments.

Firstly, in most of the experiments hitherto performed, not the tension of the fibre but the tension of the terminal tendons has been measured; for tension of fibre = tension of tendon/cos v, where v is the angle of attachment. Conversely, if we measure the degree of shortening, we find the shortening of the tendon = the shortening of the fibre/cos v. The magnitude of the possible sources of error shown by this calculation cannot be stated in simple terms, but comparison of a resting and a contracted gastrocnemius will show that, at any rate in the case of this muscle, such errors may become fairly considerable.

Secondly, before the final tension is developed in the muscle, certain displacements occur which take an appreciable time and may not be completed during a single twitch. As already mentioned, the muscle comprises several structural elements, which can be displaced in relation to each

other with more or less friction. In any steady state of the muscle there exists a condition of relative equilibrium between the stroma, the active and the inactive fibres. If this equilibrium is disturbed a certain time will elapse before it is re-established, and during this interval it is impossible to obtain any information as to the tension of the fibre. Experiments made by Gasser & Hill (1924) for other purposes may give some idea of the duration of this process of adaption. Any sudden change in the length of a muscle contracting isometrically will entail a momentary positive or negative change in tension passing far beyond the level of tension that corresponds to the new length. It appears justifiable to suppose that the time from the onset of the change of length till the new tension is reached, amounting to $^1/_{10}$—$^1/_5$ second, corresponds to the re-establishment of the equilibrium between the tensions of the structural elements of the muscle, and to their alteration of shape. So long as the disturbance of equilibrium lasts it will, as already mentioned, be impossible to say anything about the tension of the individual fibres and what takes place within them.

There is, however, another source of error, which may occur in cases where the direction of the fibres of the muscle is parallel to the direction of pull of the tendons, and which may result in errors when small bundles or isolated fibres are dealt with, namely that due to the shape of the fibres[1]. Lindhard and Möller (1930) have shown that when a conical fibre contracts isometrically, the thick end of the fibre will become shorter and thicker, whilst the thin end will become longer and thinner. Somewhere in the fibre

[1] This change of shape of the individual fibre should not however be confused with the above displacements between the structural elements of the muscle or bundle (Fischer (1934), v. Muralt (1936)).

there must therefore be a cross section which will remain unaltered during contraction, and whose position can be determined when the shape and dimensions of the fibre are known. The area of this cross section will probably determine the tension which the fibre is able to develop during contraction. If a fibre be imagined having the shape of a blunt cone, the length of which is 20 mm., its diameters being $^1/_{10}$ and $^1/_{60}$ mm. respectively, and which is able to retract to $^1/_3$ of its original equilibrium length, then it may be calculated that the unchanged cross section, which is also the one whose movement is greatest, will be situated 5.8 mm. from the thinner end when the fibre is at rest, and that during isometric contraction it will move 5.6 mm. towards the thicker end. When the change of shape begins there will be the same tension per unit of cross section area everywhere in the fibre, whilst when the change of shape is fully established there will be the same tension on any total cross section of the fibre, irrespective of its area. Thus the thicker end of the fibre will shorten and so perform work, but part of this work will result in increased potential energy in the thinner end of the fibre, which thus performs a negative amount of work. If the whole of the work was transformed into potential energy, the change of shape would not entail any heat formation and would not involve any loss of tension; but that this is not so is illustrated by a simple example.

Imagine two elastic cords of the same material and the same length but with different cross sections, so that their tensions when they are drawn out to twice their length will be in a ratio of 1:2. The two cords are fixed so that their ends will meet when each is stretched to twice its original length. If they are joined together in this position and then

allowed to pull against each other, it will be seen that the thicker cord will shorten and the thinner one will be extended until the tension between the two fixed points is the same everywhere. The increase of tension, x, of the thinner cord will then be determined by the equation $1 + x = 2\,(1 - x)$, or $x = \frac{1}{3}$; at the same time the increase of length (a) in the case of the thinner cord if it is perfectly elastic, will be $a = \frac{1}{3}$. The increase in potential energy of the thinner cord will then be

$$E_1 = \int_0^a (1 + x)\,dx$$

and the loss of energy of the thicker cord

$$E_2 = 2\int_0^a (1 - x)\,dx$$

which, when $a = \frac{1}{3}$, gives

$$E_1 = \frac{7}{18} \text{ and } E_2 = \frac{10}{18},$$

which again gives the total loss of energy of the system $= \frac{1}{6}$.

Reference may be made to the original paper (1930) for the method of calculation employed. It will suffice, in order to indicate the order of the results found, merely to state that the calculation made in three cases assuming a maximal tension of 3 kg./cm.^{2} gives an average deformation-heat $Q = 0.0109$ cal./cm.^{3}.

It is obvious that these computations of the loss of energy in deformation cannot more than approximately indicate the order of magnitude, as the fibres *in situ* are unable to move independently of the neighbouring ones, and because Q will vary with the shape and dimensions of the fibres.

But it is evident that the source of error referred to here may under certain circumstances make the result of the myothermal experiments performed in the usual manner untrustworthy since the ratio between tension and development of heat becomes variable.

The muscles of the living animal have partly a static, partly a dynamic function; they must either maintain a steady tension or perform an amount of positive or negative work. As maintainance of a steady tension and the performance of work are essentially different from a mechanical point of view, and as the process taking place in the muscle, or rather the muscle compartments, must be supposed to be the same in all cases, these functions should be considered to arise from an underlying, primarily mechanical change in the muscle, and the change of elasticity previously referred to then naturally suggests itself.

A. Fick first maintained that the contracting muscle was a new elastic body, but knowledge of muscular function was not sufficiently advanced at that time as to make it possible for him to prove his assertion. Later on A. V. Hill, on the basis of his myothermal experiments, maintained the same view, for he claimed to be able to demonstrate that when an isometrically contracting muscle has developed maximal tension, the muscle is able to perform an amount of positive work without further heat production. Hill therefore maintained that the primary mechanical function of the muscle was the development of tension (potential energy) and that the fully developed tension may then either be dissipated as heat or be employed for work, according to the external conditions, without influencing the consumption of energy by the muscle. Therefore Hill defined the mechanical efficiency of the muscle as:

$$\frac{\text{(Potential Energy thrown into an active Muscle by Excitation)}}{\text{(Total Chemical Energy liberated as Heat)}}.$$

These ideas agree very well with the conception that, when stimulated, the muscle will assume a shorter length of equilibrium; if fixed at its original length it will thus be able to develop tension, but if allowed to shorten, it will be able to perform some work. Hill's interpretation and valuation of his previous results appear to have undergone some change. In his more recent publications it seems, however, as if Hill regards his original view as incorrect, that the muscle when it shortens and performs an amount of work must develop extra energy in addition to that released by the stimulation (Fenn Effect). The number of phases recognized in the heat production have increased in recent years and their interpretation under different experimental conditions becomes very complicated. It seems to us almost hopeless to attempt such an analysis in the case of tetanic muscular contraction, the complex nature of which we have previously made clear. Hill's conception of the mechanical function of the muscle is, however, untenable as will appear from the following investigations into tensionless contraction.

If a frog's muscle, e. g. a Gastrocnemius, is immersed in Ringer's solution so that it does not support its own weight, and is then submitted to a series of indirect stimuli, the muscle will not develop mechanical energy. The first stimulus will cause it to change its shape, but then, provided the stimulation is of a suitable frequency, it will remain almost completely at its position of equilibrium. It is unable to shorten further, and is thus unable to perform any work, and it cannot develop tension at its length of equilibrium. Thus the stimulus does not bring about develop-

ment of potential energy in the muscle. Only if some external force prevents the muscle from assuming its length of equilibrium will it develop tension. Thus the idea of efficiency as a physiological characteristic of the muscle fibre vanishes. It might perhaps be imagined that a momentary development of mechanical energy took place in the muscle and that such energy would also immediately be dissipated as heat. But this is not so. The contraction without tension involves only a very slight development of heat. A slight heat formation will take place because in practice it is impossible to make the contraction absolutely tensionless. Any muscle has a characteristic shape corresponding to its length of equilibrium when unstimulated. Near the length of equilibrium the shape is determined by the fibres, whereas in greater changes of length the stroma no doubt plays a part both in the case of extension and contraction. Thus it is not unusual to see the perimysium fold during maximal contraction. The single fibre, too, possesses a typical shape, which, apart from internal forces, is determined by the sarcolemma. The usual stage of restitution is also present only to a slight extent after tensionless contraction, if it occurs at all.

ASMUSSEN has measured the difference between the development of heat after a series of isometric tetani and corresponding number of tensionless contractions; he employed for this purpose a thermo-couple consisting of about 250 insulated copper-constantan junctions, half of which were placed in a muscle chamber filled with Ringer's solution but not in direct contact with the muscle, and the other half placed in ice water stirred by means of ice-cold air. A Hartmann-Braun galvanometer was employed as measuring instrument. The result of a series of experiments is repro-

duced in Fig. 53. The method is only an approximate one and can hardly give absolute values of the heat production; it seems, however, sufficient for this purpose, where the object is to differentiate between the heat production in the case of contraction of a loaded muscle and of an unloaded muscle, that is, between isometric contraction and tensionless contraction. Asmussen's results correspond well with the heat measurements made on muscles under varying loads by McKeen Cattell (1932). Since, however, the theory and principle of tensionless contraction has not been

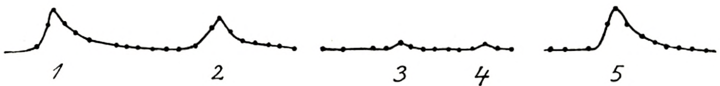

Fig. 53. Total heat production of a frog's muscle. Arbitrary units. 1 and 2 isometric tetanus. 3 and 4 tensionless contraction. 5 as 1 and 2.

previously fully discussed, and as it plays a decisive part in the theoretical interpretation of the contraction phenomena, we considered it desirable to test Asmussen's experimental results by other methods. With this end in view A. Topsøe-Jensen has made a series of determinations of the oxygen consumption of the muscle under isometric tetanus and under tensionless contraction. The experiments were made with small frog's Sartorii (weight 35—65 mgm.) by means of Krogh's micro-respiration apparatus (1914), the principle of which is well known. The muscle was placed in a chamber consisting of a glass-tube (volume about 7 cm.^{3}), one end of which was closed and which was provided with a side tube with a glass stopper, into which a couple of platinum electrodes were sealed. Beneath the latter, also attached to the glass stopper, was a small celluloid pot in which the muscle was placed in Ringer's solution saturated with oxygen. By means of two small hooks the muscle could be

fixed at its length of equilibrium when the nerve was placed across the platinum electrodes. The CO_2-absorbent (5 per cent. KOH) was spread on filter-paper covering the interior of the muscle chamber. During the experiment the muscle chamber and the control container were placed in a water-bath at room temperature. In order to avoid changes due to temperature variations, the manometer was read by means of a kathetometer, and by blank experiments it was ascertained that the stimulus alone did not influence the manometer. As it appeared that the manipulations required in making the preparation resulted in an increased and highly fluctuating basal (resting) metabolism, the mounted preparation was placed for 15—30 minutes before the experiment in oxygenated Ringer's solution. The muscle was then first stimulated without tension for 15 sec. with a maximal tetanizing current and the manometer read every 10 minutes for 30—40 minutes. The muscle was now secured isometrically and the whole experiment repeated precisely as in the case of contraction without tension. The readings of the manometer were constant in all cases after the cessation of the experiment. The results are given in Table VIII.

As will be seen, the results of these experiments agree approximately with those of Asmussen's. In the experiments of the 6th and the 13th the increase of the O_2-absorption in tensionless tetanus is strikingly large; it should, however, be borne in mind that errors of this magnitude are likely to occur, if the arrangement of the muscle is not quite successful, if for instance there is too little fluid on which the muscle may float, or if it adheres to the container and so on, though errors in the other direction can hardly occur. The lowest figures of the column must therefore be considered the correct ones. The increase of the oxygen absorption, in

Table VIII.

Date	O_2-consumption in mm.³ per gm./min., determined in 10 min.-periods				Total rise of O_2-consumption per gm. of muscle in 30 min.	
	Rest	Tensionless Tetanus for 15 sec.	Rest	Isometric Tetanus for 15 sec.	Tensionless Tetanus	Isometric Tetanus
6/x	1.53	1.73	1.53	3.57	5.03	31.6
8/x	1.70	1.80	1.70	3.30	1.01	30.0
9/x	1.28	1.44	1.28	3.08	1.59	27.0
12/x	1.02	1.23	1.02	2.25	2.04	26.6
13/x	1.36	1.64	1.36	2.73	4.09	19.1
14/x	1.04	1.25	1.04	2.40	2.08	26.0
15/x	1.68	1.96	1.68	2.66	2.80	15.9

the course of isometric contraction must, of course, vary according to the size and condition of the muscle. It will moreover be noticed that whilst the increase of metabolism after tensionless tetanus has ceased at the end of 10 minutes in all the experiments except the two specially referred to above, it takes much longer however after isometric tetanus.

From the results of the two preceding series of experiments, showing that the metabolism of the muscle is only slightly influenced by the tensionless contractions, it must be supposed that such contractions would less easily give rise to fatigue than contractions during which the muscle develops mechanical energy. This proves to be so. If, for instance, two gastrocnemii of the same frog are placed in a myograph and one of them is stimulated a certain number of times without loading, and then both muscles are loaded and stimulated simultaneously to fatigue, fatigue occurs practically at the same time in either case, as is shown by Table IX (unpublished experiments by A. Topsøe-Jensen).

For purposes of comparison it should be mentioned

Table IX.

Date	Shortening in arbitrary Units			Tensionless Contractions	Contractions under Loading		Diff. b — a	Decreased Working Capacity $\frac{(b - a) \cdot 100}{b}$
	Beginning		End					
	a	b	a and b	a	a	b		
4.12.36	40	40	3	180	500	620	120	19 Gastrocn.
14.12.36	39	44	3	300	480	580	100	17 —
18.12.36	38	38	3	360	455	470	15	3 —
19.12.36	38	38	3	420	485	495	10	2 —
23.12.36	46	47	3	180	400	500	100	20 Semitend.
23.12.36	44	43	3	240	515	505	—10	—2 —

that in 16 double experiments with symmetrical muscles which were stimulated to fatigue in the same manner as before, but without preceding tensionless contractions, an average difference between the number of contractions of 3.6 per cent. was found, with an average error of 54 per cent.

A closer examination of the table will show that at the beginning of the experiment the two muscles gave the same height of contraction; only in the experiment of Dec. 14th has the control muscle an appreciably greater height of contraction than the (a)-muscle. This experiment is therefore of less value, because we do not know whether the difference in the last column is due to fatigue of the (a)-muscle owing to the tensionless contractions, or whether it is due to different development of the two muscles. The experiments are stated chronologically and it therefore appears as though the highest values in the last column might be due to lack of experience in the experimental technique. The number of tensionless contractions preceding the fatigue experiments proper is apparently of no importance. In the experiment of Dec. 19th, in which the number of tensionless contractions almost equals the number of loaded contractions, required to bring

both muscles to the limit of absolute fatigue, the effect of these contractions is scarcely demonstrable. The result of these experiments is thus directly opposed to investigations by RIESSER & SCHNEIDER (1929), who maintain that the time of onset of the fatigue is independent of the loading of the muscle.

The general impression of the three series of experiments referred to is that tensionless contractions are without appreciable effect on the muscle, which in turn implies that in the course of the tensionless contraction no irreversible processes take place to any appreciable extent. Therefore it must be supposed that the energy liberated by the stimulus forms part of a cyclic process, the last phase of which is the re-establishment of the excitation—and contraction potentials. This energy moreover must be supposed to be equal to the work which the fibre would be able to perform, or rather the potential energy it would be able to develop, if it was fixed at its original length of equilibrium at the moment when it changes from one elastic condition to the other, and which must be of the same magnitude as the electrostatic forces keeping the molecular chains of the myosin micellae (see below) extended in the "state of rest". The tensionless contraction, which is the direct result of the stimulation when the muscle is uninfluenced by external forces, should then chiefly take the course of a series of reversible processes. As a picture of the mechanism of the energy output we may imagine a pendulum that is supported above its lowest position. It will then possess potential energy. If the pendulum is released its potential energy will disappear but simultaneously it will obtain an equivalent amount of kinetic energy which reaches its maximum at the lowest position of the pendulum and disappears again

under the development of potential energy as the pendulum moves on to the end of its swing in the opposite direction. But if external resistance stops the movement the kinetic energy will be transformed into heat. This analogy may be extended to the muscle fibre. If, as a consequence of the change of elasticity, the latter performs work or develops potential energy, which is dissipated irreversibly into heat, the regeneration of the above-named potentials will require the liberation of an amount of energy equal to that which the fibre has lost in the shape of work or heat. As the tensionless contraction must no doubt be considered very rare under natural conditions, we generally find a relatively long-continued stage of recovery after the contraction, during which a number of energy-producing processes occur, which besides yielding the energy required for the regeneration of the contraction potential also dissipate heat; of these processes the essential one seems to be the oxidative destruction of carbohydrate. v. MURALT's (1935) statement that "Das Funktionsziel der Muskulatur ist die Bereitstellung mechanisch verwertbarer Energie aus chemischen Energiereserven", and that "Das Endglied des Energieumsatzes ist primär immer 'die Bereitstellung', denn der eigentliche Umsatz vollzieht sich zunächst so, dass potentielle, mechanische Energie aus chemischen Energiereserven entsteht", would appear to be theoretically untenable. It is correct that the primary function of the muscle is "Bereitstellung mechanisch verwertbarer Energie"; but this "Bereitstellung" is expressed by the change of equilibrium length, the only mechanical process taking place in the muscle fibre as a direct consequence of stimulation. All other mechanical phenomena, work, development of tension etc., are secondary, a consequence of the interference of external forces.

If, on the basis of the investigations into tensionless contraction, the histology of muscle, its minute structure, and into the electrical phenomena of the fibre, (supported especially by H. H. Weber's, Kurt H. Meyer's and our own experiments), we try to form a picture of the internal condition of the "resting" and the "contracted" fibre, to establish a contraction hypotheses, we arrive at the following result:—

The rod-shaped formations in the *A*-segments of the muscle fibrils consist of myosin micellae composed of long, flexible but firmly connected molecular chains, which in a state of equilibrium are completely deranged, coiled or collapsed. K. H. Meyer attaches great importance to the fact that the individual links of the molecular chains are connected by means of very firm valencies; if these are lacking the orientated atoms and molecules may become disorientated through a rotation that does not change the shape of the object, as is the case with dipoles orientated in a field when the activity of the field ceases. In the unstimulated resting muscle fibre these molecular chains are, however, not in their state of equilibrium, but are more or less straightened in an electric field. They cannot be completely straightened, for, if so, the *A*-segment of the fibre would behave very much like a tendon, i. e. it would be near its limit of elasticity and, therefore, unable to withstand further stretching without the occurrence of irreversible structural changes; the micellae cannot be completely coiled either, for the *A*-segments would then be unable to shorten. K. H. Meyer does not take the electrostatic forces in the fibre into account and assumes that when a muscle is at its state of elastic equilibrium the contractile elements, the myosin micellae, must be in a similar condition. He maintains that the molecular chains that are stretched in the case

of passive extension will coil up when the latter ceases, whereas in fact though the stretched molecular chains do become folded to some extent, they do not completely reach the coiled-up state of equilibrium which they would assume if left alone.

When the muscle fibre is stimulated a sudden large change in the excitation potential (i. e. the potential difference between end-plate and fibre substance) is seen, as referred to above, which is followed by a fall in the fibre potential; and thus a sudden, marked decrease might occur of the electric field keeping the molecular chains of the myosin micellae stretched, so causing them to "collapse". The completely coiled-up or collapsed state of the molecular chains is, however, unstable, as the electric potentials are soon re-established. This, as we have seen, causes the molecular chains to become more or less straightened again. According to the investigations into the minute structure of the myosin previously referred to, compared with similar work on rubber and elastic connective tissue, it must be supposed that when the molecular chains are straightened to the state of longitudinal orientation a decrease of the entropy of the system will occur, whilst collapse is accompanied by an increase of the entropy. Therefore it is conceivable that the work required for the re-establishment of the electric field originates from the increase of entropy. The condition of the myosin micellae in the "resting" fibre is thus determined by an interaction between elastic and electrostatic forces in the fibre, and corresponds to the condition of the molecular chains in the passively extended rubber cord. The stability of the condition depends on the stability of the electric field. On the other hand the maximally contracted muscle fibre corresponds to the resting rubber

cord, the molecular chains of which are completely coiled up; but in the case of the muscle fibre this condition is unstable owing to the rapid re-establishment of the electric field.

It is not known what substances determine the presence of the electric field. There is, however, reason to believe that somewhere at a boundary surface in the muscle compartment an ion production and exchange takes place. The strength of the field may vary, partly as the result of the rate at which the ions are produced, partly owing to variations in the permeability of the "membrane" itself, and partly owing to variations in the rate of the process by which the ions are removed. It is known that the strength of the field may remain practically constant for 60—70 minutes; but it is probably not always of the same intensity; on the contrary no doubt like all other natural phenomena it varies under changing conditions. But for the time being, we can only advance hypotheses on this point.

The mechanism of contraction outlined above applies to tensionless contraction. If external forces prevent or hinder the collapse of the myosin micellae the muscle fibre will develop tension, also termed potential mechanical energy, and this development of energy will continue till the field strength, decreased as a consequence of the stimulus, has regained its former intensity. In this case the regeneration of the electric field must take place by means of other forces than in the case first considered, the reversible process now being transformed into an irreversible one, which entails the occurrence of oxidation processes in the fibre; but the mechanism involved is at present unknown. These processes, whose purpose must be the regeneration of the potentials of stimulation and contraction, are not reversible

but entail a loss of energy to the fibre, which is manifest as dissipated heat corresponding to the mechanical energy developed.

Tension will arise in a muscle when the contractile elements are prevented from assuming their length of equilibrium, and only in that manner. In the living organism the muscles are usually extended beyond their length of equilibrium and, therefore, they possess tension; this tension is termed muscle tonus. The tonus may vary either as a consequence of external forces causing changes in length of the muscle, or more especially owing to variations of intensity of the electric field in the muscle fibre produced by stimulation, and finally owing to variations in the intensity of the field of the unstimulated fibre. The last-named possibility is for the time being purely hypothetical, for variations in muscle tonus can be explained on the basis of the somatic innervation alone.

If, on the other hand, the individual fibre is considered, it has, as referred to above, only one degree of contraction in indirect stimulation and therefore its tonus cannot be graded as a consequence of somatic innervation, less so because this innervation can produce changes in one direction only. If the tonus of the individual fibre could be graded and vary in either direction these variations might be due to changes of intensity of the electric field, which controls the length of the myosin micellae and thus the length of equilibrium of the unstimulated fibre. Variation of the field not caused by the somatic innervation has not been demonstrated directly but must be considered probable; and the extent of such variation may become very large, covering the whole scale from completely coiled-up to perfectly straight myosin micellae. This aspect of the mechanism

of contraction will not, however, be dealt with in detail here, since there is insufficient experimental work to substantiate it thoroughly.

In this connection mention may be made of the considerable number of experiments and arguments on the basis of which it has been maintained that muscular tonus is regulated through the autonomic nervous system; whilst according to another view the tonus is regulated exclusively by means of the somatic innervation. The latter has been demonstrated in resting muscles. Neither of these two attempts of an explanation is, however, complete, but on the basis of the hypothesis of contraction advanced above it is possible to combine the two viewpoints so as to enable us to explain the existing experimental results if it is supposed that the equilibrium length of the unstimulated fibre is regulated through autonomic nerves, whilst it is the somatic innervation which determines the tension superimposed on this background. For the time being such a hypothesis lacks experimental support.

If the working hypothesis outlined here is to be actually applied to the process of muscular contraction it is, of course, necessary that the electrostatic forces existing in the muscle compartment are of the order necessary to keep the myosin micellae extended to the degree they possess in the A-substance of the resting, unstimulated fibre. As the latter may be stretched on extension and resume its original length by relaxation, the molecular chains cannot be completely straight when the fibre is at rest, as is the case for example in a tendon; complete straightening must be supposed to correspond to a degree of extension of about 1.4 $\times$ the resting length, or to about 50 per cent. elongation of the A-substance. Further extension gives rise to irreversible

changes in the structure of the fibre, as mentioned above. On the other hand we may expect the maximal degree of shortening of the *A*-substance to be to about $^1/_4$ of its resting length. As the electrostatic forces correspond only to the degree of contraction of the muscle fibre at its resting equilibrium length and not to the maximal contraction of the muscle, an idea of the relations of mechanical and electrical energy can be arrived at, if we examine the tension-length curves previously referred to. Before dealing with this question we shall, however, see what can be learned about the electrical forces by examination of the electrostatic conditions of the fibre.

The electrostatic forces acting on the contractile elements of the muscle fibre play a prominent part in excitation and contraction processes; but direct measurement of these is so untrustworthy at present that we must be content with relative values of their sizes in different parts of the fibre. Estimation of the order of magnitude of the electrostatic energy of the system involves not only a knowledge of the potentials, but also determination of the resistance and capacity existing in the fibre.

Considerable changes in experimental technique resulted from the realization that in the understanding of electrical phenomena in muscle fibres, the electrostatic potentials were at least as important as the currents arising from them. The electrostatic methods of measurement in this field have been developed by Buchthal, who has stated their principles and limitations, and has examined the sources of error which may arise under different conditions. There is no need, however, to follow this development in detail here; if required, reference may be made to the published accounts, but a brief description will be given of the apparatus

which at present is considered suitable for this work and of the precautions that must be taken if reliable results are to be obtained by its use.

With the introduction of amplifying valves into electrophysiology it became possible to detect far smaller potentials and potential changes than previous methods allowed. By combination of the amplifying valve with the oscillograph as a recording instrument, more rapid changes in potential could be followed and their correct time-relations preserved. For an account of the comprehensive literature on the subject reference may be made to Buchthal & Nielsen (1936).

Examination of the electric phenomena in the individual cell makes great demands on the sensitivity of the measuring instrument, but moreover other precautions are required which can be ignored in experiments on nerves and whole muscles. Thus, when single cells are used, care must be taken to prevent any appreciable flow of current in the tissue, or to the measuring instruments employed. In order to determine the potentials actually existing, care must be taken that the measuring instrument has a resistance that is very large in proportion to the preparation, and since this and the leading-off electrodes have an internal resistance of several million ohms, the amplifier must have an input resistance of at least 100 meg. ohm, if we are to be certain not to take any appreciable current from the preparation.

The apparatus employed must be able to reproduce exactly not only potential differences of resting systems (e. g. the resting potential of the muscle fibre) but also rapid and slow changes of potential. All these requirements can be fulfilled only by the use of a direct current amplifier. As recording instrument either a mirror oscillograph can be employed, or—as we have done—a cathode ray oscillograph.

Owing to its high input resistance this instrument is especially suited for use in conjunction with valve amplifiers; moreover it possesses negligible inertia and is not damaged by overcharging, a property that makes it especially well-suited for measurements on tissues stimulated electrically.

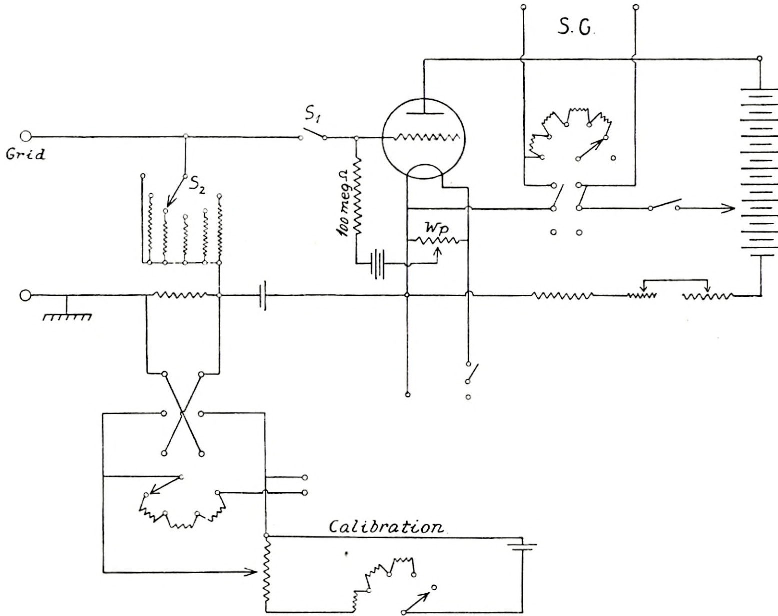

Fig. 54. Electrostatic electron tube voltmeter. Wp = Compensation of the constant component of grid current. S_1 = Control of grid current compensation. S_2 = Variable shunt resistance to the preparation, 1 meg. Ω —100 meg. Ω. $S.\,G.$ = String galvanometer. (BUCHTHAL).

In their first experiments BUCHTHAL & PÉTERFI (1934) worked with a Binant electrometer; and in recent experiments, in order to follow more rapid potential changes, BUCHTHAL has also employed an electrostatic valve-voltmeter in connection with a string-galvanometer for recording. In this arrangement (Fig. 54) the disturbing effect of the grid current was eliminated. The grid current of the input

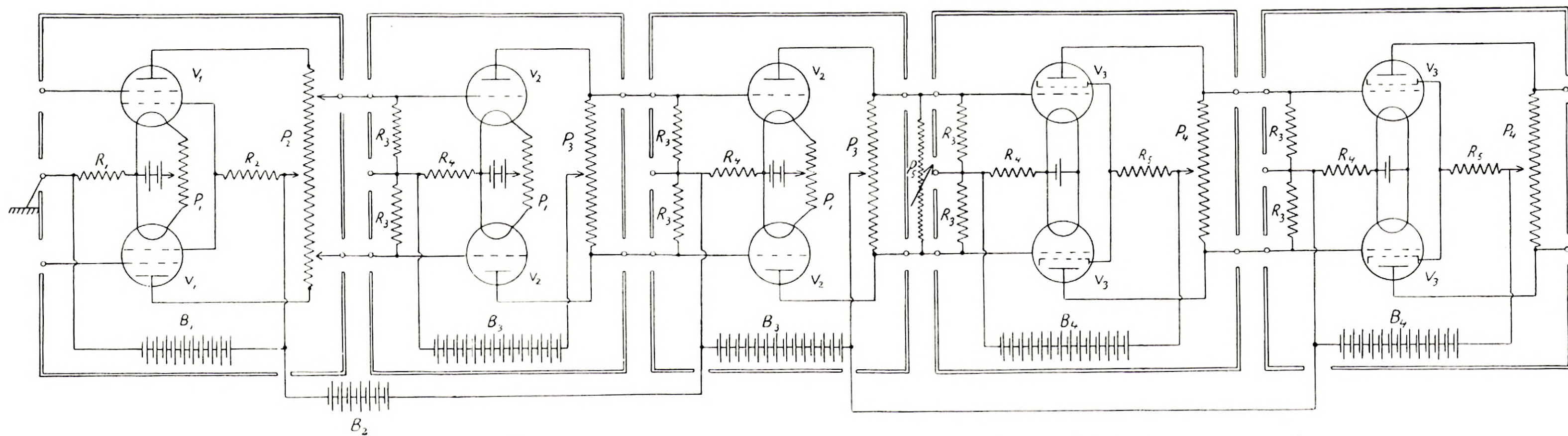

Fig. 55. Electrostatic balanced D. C. amplifier. (BUCHTHAL & NIELSEN).

$R_1 = 1000\ \Omega$.	$P_1 = 25\ \Omega$.	V_1 = Osram T 113.	B_1 = 20 Volt.
$R_2 = 3000\ \Omega$.	$P_2 = 40000 + 1000 + 40000\ \Omega$.	V_2 = Telefunken RE 034.	B_2 = 70 Volt.
$R_3 = 2$ Meg. Ω.	$P_3 = 100000 + 10000 + 100000\ \Omega$.	V_3 = Philips KF 1.	B_3 = 120 Volt.
$R_4 = 500\ \Omega$.	$P_4 = 200000 + 50000 + 200000\ \Omega$.		B_4 = 180 Volt.
$R_5 = 0.2$ Meg. Ω.	$P_5 = 5000\ \Omega - 1$ Meg. Ω.		

valve makes it impossible to determine the actual rise of potential differences and may cause changes in the resistance of the preparation to be mistaken for potential changes. By employing a special valve with a carefully isolated grid coil it was possible to eliminate the inconstant compo-

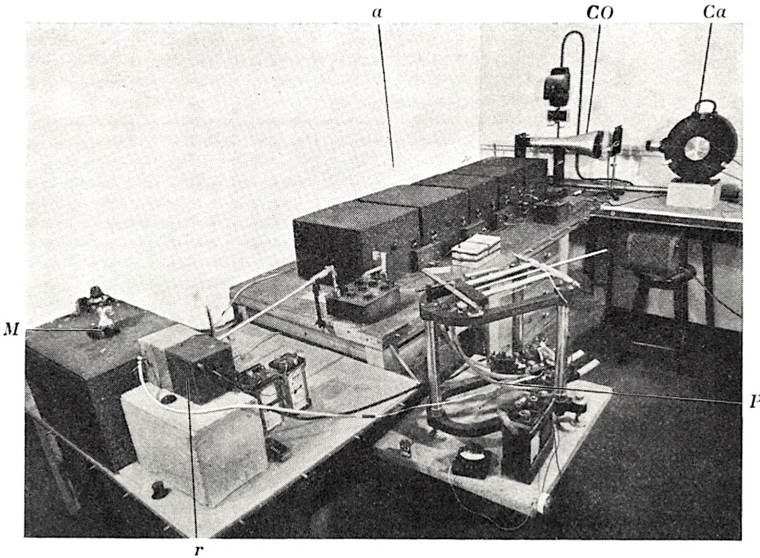

Fig. 56. Arrangement for measuring potential differences on single muscle fibres. M = microscope, micromanipulator with micro-electrodes and preparation. r = relay to prevent stimulus escape. P = Helmholtz-pendulum. a = D. C. amplifier. CO = Cathode ray oscillograph. Ca = camera.

nent of the grid current and to compensate the relatively constant ion-current to such an extent as to make measurements without current-flow possible. Compared with the "electrometer" valves usually employed, this arrangement has the advantage that it gives greater amplification, so that in many cases such an arrangement with a string galvanometer as recording instrument will be adequate. The use of the string galvanometer will, however, cause the period of inertia to become comparatively long. Therefore, in order

to record very rapid potential changes e. g., change of the excitation potential and its time relations with the contraction potential, it was necessary to build a balanced direct current amplifier with electrostatic input (Fig. 55 and 56). The variations in voltage of the batteries was reduced to a minimum by balancing of the stages individually and also with respect to each other (negative direct current feed-back). This arrangement proved to be extremely stable both to sudden and to slow variations of voltage in the current.

After "warming up" the displacement of the base line was only 10 mm. (= 100 microvolt) in the course of three hours. Such stability is necessary in many biological measurements because we are often forced to work near the limits of the amplifying arrangement. The theoretical limit of potential measurements in a conducting system is where the potential changes which result from the heat-motion of the electrons become of the same order of magnitude as the potential differences which it is desired to measure. The size of these disturbing voltages depends on the temperature and the resistance of the conducting elements, whilst the disturbances in the output circuit of the amplifier depend on the degree of amplification and the range of frequency response of the arrangement. The latter disturbances can be calculated according to the formula

$$V = 2\sqrt{K \cdot T} \cdot \sqrt{R \cdot F} \cdot G$$

where V denotes the magnitude of the disturbing voltage at the output terminals of the amplifier, K Boltzmann's gas constant ($1.37 \cdot 10^{-23}$ Ws per degree of absolute temperature), T the absolute temperature, R the resistance of the object, F the range of frequency covered by the measuring arrangement, and G the degree of amplification.

In the case of a frequency range for instance of 10,000 cycles and a resistance of the object of 2 meg. ohm, the active "disturbance-level" will become 18 microvolts. The frequency response curve of the amplification arrangement employed by us will appear from Fig. 57. It will be seen from the figure that the degree of amplification is constant up to frequencies of 10,000 cycles. Knowledge of the theo-

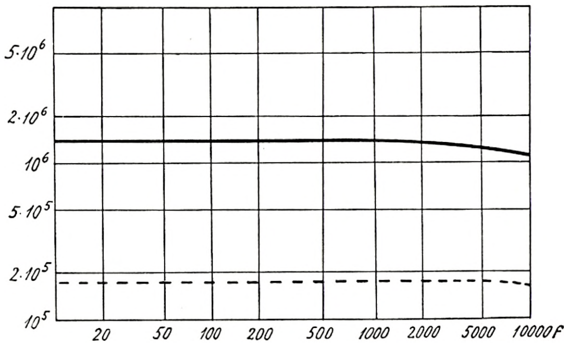

Fig. 57. Measured amplification for different frequencies. Upper curve 5 stage amplification. Lower curve 3 stage amplification. Ordinate: amplification in volts. Abscissa: frequency cycles per sec. (Buchthal & Nielsen).

retical and practical limitations of the method employed is, however, not sufficient to safeguard against error in biological work; it is constantly necessary by means of control experiments to safeguard against potential changes occurring from various sources of error such as injury to the preparation, mechanical displacement of the electrodes, disturbances of capacity, "stimulus escape" and so on as referred to in detail in the various papers.

Finally, attention should be called to the fact that the potentials that are measured even under the best possible conditions are only summation phenomena originating, among other things, from diffusion potentials, absorption

potentials, oxido-reduction potentials which at present cannot be analyzed, but which may be considered as indicators of what is taking place in the cell. Such potentials may well be important factors in biological processes. The difficulty of measurement and of reproducing the experimental conditions of experiments, when whole muscles are used, has often resulted in the formation of poorly supported hypotheses, because it has been impossible to establish the real connection between the measurements and the structural and functional properties of the tissue and this has caused more cautious observers even to question the existence of potential differences in resting systems, which however is now beyond doubt. The potential differences observed must, however, as already mentioned, be considered a reflection of a "dynamic equilibrium" between formation and removal of ions.

In the first publication on the electrostatics of the muscle fibre Buchthal & Péterfi (1934), used frog's muscles as experimental objects, especially the broad abdominal muscles, which were examined mostly in situ, the circulation being intact, sometimes in isolated preparations of thin muscle plates. It proved to be of considerable importance that the muscle fibre should be actually exposed; if the current was led off to the electrometer through the peritoneum or other connective tissue, the fibre potentials obtained were smaller than if leading off took place from the sarcolemma. On the muscles of the extremities the potential differences could not be demonstrated at all if the electrodes were placed on the fascia of the muscle. The sarcolemma, too, has insulating properties. The potentials measured become far less when the electrodes are placed on the sarcolemma than if they are inserted into the fibre substance itself

(Fig. 58). It appeared, however, that the potential differences measured through the sarcolemma were of the same type as those obtained by leading off from the fibre substance itself; they gave a quantitatively diminished but qualitatively correct expression of the resting potential of the fibre. It became preferable in later work to use the relative (epilemmal) potentials because the unavoidable injury to the fibre in the case of sublemmal measurements entails considerable uncertainty, as unless the injury to the tissue is quite the same at both electrodes, unbalanced injury potentials will be produced. As moreover the insertion of the electrodes into the fibre substance brings about a microscopically demonstrable disturbance of the cross-striation, it will be impossible to obtain normal values, even though an average figure might be arrived at by increasing the number of experiments performed. However, the potentials determined will always be too small.

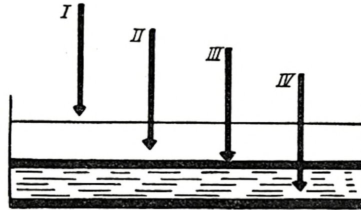

Fig. 58. Different positions of electrodes. I. Above the preparation. II. In Ringer solution. III. On the sarcolemma. IV. Inside the fibre. (BUCHTHAL & PÉTERFI).

The principal result of the experiments was that when both electrodes were placed on the surface of the same fibre, a potential difference was found which, in contradistinction to that found in similar examinations of other organs, varied in a fairly regular manner with the mutual distance of the electrodes. The potential increases with the distance between the electrodes to a maximum (Fig. 59), the latter presumably implying that the electrode moved has either passed the place where the fibre ends, or has slipped on to another fibre. Owing to the difficulties which may be associ-

ated with the following of a single fibre in situ, the distance between the electrodes should not be greater than about 500 μ. Fairly large individual variations are, however, found in different fibres. Fig. 59 and Table X give some examples of the experiments.

The first column of the table shows the distance between

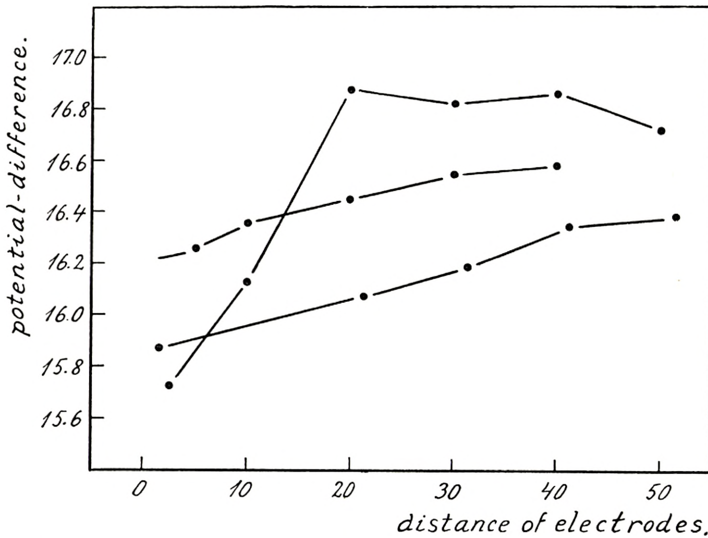

Fig. 59. Potential differences in the frog's muscle fibre with electrodes different distances apart. (Buchthal & Péterfi).

the electrodes, the figures representing units of 100—150 μ, 0 indicating that both electrodes are in Ringer's solution. The next 9 columns show the variation of the potential with the distance between the electrodes. The direction of the variation depends on whether the earthed or the non-earthed electrode has been moved. The figures given represent scale divisions, each division corresponding to 0.5—1 mV. For purposes of comparison it may be stated that the average of 16 sublemmal measurements is 45 $\pm$ 8.4 mV, corresponding to a distance between the electrodes

Table X.
Dependence of the Resting Potential on the Distance between the Electrodes.

Distance between Electrodes	Experiments								
	I	II	III	IV	V	VI	VII	VIII	IX
0	16.00	..	16.00	16.15	14.20	15.72	16.90	16.00	..
1	15.98	16.10	17.00	16.35	14.70	16.73	16.70	17.90	16.00
2	15.90	15.97	17.20	17.16	16.30	16.80	16.50	17.95	17.90
3	15.85	15.65	17.30	..	16.55	16.82	16.65	16.55	17.95
4	15.80	15.45	16.10	..	..	16.87	17.65	15.93	16.55
5	15.60	15.50	..	..	..	16.72	18.10	..	15.93

of 100 μ, which shows that these measurements are subject to considerable errors, as already stated. When the fibre looses its excitability the fibre potential will disappear; in dead fibres no definite potential differences are found, no matter what the distance between the electrodes. In muscles poisoned by chloroform the potential will decrease gradually as the intensity of narcotisation increases. As previously mentioned, it will also disappear when influenced by radium, whilst it remains uninfluenced by curare.

It was to be expected that potential differences would be found between the different structural elements, for example, between the sarcolemma and the contractile substance or between the nuclei and the sarcoplasm of the muscular cell, but such potential differences do not change with the distance between the electrodes. This variation of the fibre potential cannot be due to movements of the electrodes as shown by control experiments, nor can it be explained by conduction through the surroundings, since it is the same whether the preparation is covered with Ringer's solution or with paraffin. Therefore it must be considered

that the variation of the potential difference with the distance between the electrodes is associated with the segmental structure of the fibre. This view is further corroborated by the fact that when the electrodes are placed on unstriated muscle cells a potential difference may be demonstrated, but it does not vary with the mutual distance between the electrodes. For this reason we must believe that the individual muscle compartments are electrically isolated from each other, and that the boundary may perhaps be Krause's membrane. It is this membrane which determines the cross-striation; if it is disturbed, the cross-striation is also disturbed; in that case the excitability of the fibre will cease and the resting potential will disappear. Thus it plays an all-important part in the life and function of the fibre, but apart from the above, the underlying histological structure and its histo-chemical, or physico-chemical properties are unknown. On stimulation, either the rapid change of the excitation potential or the direct application of an electric current, results in a change of the fibre potential which is re-formed in the following recovery period. Buchthal & Péterfi showed that during contraction the fibre potential was diminished by 2—5 millivolt (Fig. 60) and that it was only slowly re-established after cessation of contraction; but with the apparatus then employed the authors were unable to follow the change itself at the moment of stimulation. Buchthal later succeeded in doing so (1934). It was first ascertained that the fibre potentials and their fluctuations could be measured directly, or that the resting potential

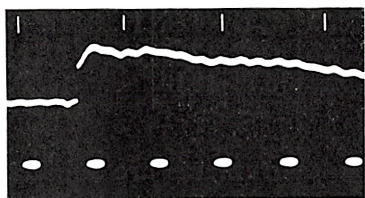

Fig. 60. Frog's muscle fibre directly stimulated. Decrease of the *F—F* potential Time marker 20 msec. (Buchthal).

could be compensated and then possible fluctuations of it recorded. According to the position of the electrodes on the fibre and the distance between them, the resting potential is from 4 to 10 mV, and decreases when the circulation in the muscle ceases, e. g. in decapitated frogs in which the spinal cord has been destroyed. As in such cases the circulation will cease first in the abdominal muscles, these will at a certain stage have a lower potential than the muscles of the limbs. The resting potential is also diminished in poisoning with cyanide and on continued stimulation. When the fibre is stimulated the resting potential will suddenly fall 1—3 mV, usually returning to its original value in 0.5—2 sec.

Sometimes the regeneration may, however, take much longer. The time course of the potential change shows that it has nothing to do directly with the excitation-potential; the latter change may sometimes be observed as a slight irregularity of the first abrupt part of the contraction potential. The combined curves obtained by simultaneous recording of both changes have previously been referred to.

In order to obtain further information as to the nature of the fibre potential, Buchthal & Lindhard (1936) examined the potential changes occurring with variations of temperature. The potential was measured and recorded as already described. The preparation was placed on a vulcanite capsule resting on a very thin cover-glass (Fig. 61). The capsule was provided with two short, vulcanite tubes by means of which water was led through the interior of the capsule from a Mariotte's flask. There were three of these, one containing water at 5—10° C., the second water at 12—18° C., and the third water at 23—28° C. As it was found that the temperatures inside the capsule, and of the preparation, might differ to a considerable extent from

each other, a thermo-couple consisting of a carefully insulated copper-constantan junction was placed on the preparation, in the immediate neighbourhood of the microelectrodes; the other junction was placed in a Dewar-flask

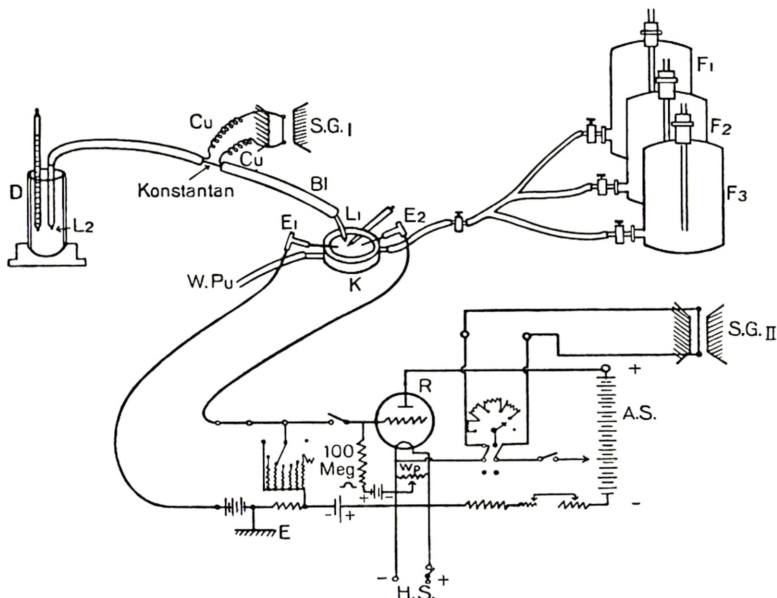

Fig. 61. Arrangement for measurement of the effect of temperature on the potential differences.
F_1, F_2, F_3 = Reservoire with water at different temperature. K = Capsule with electrodes E_1 and E_2 and thermo-couple L_1 to measure temperature of the preparation. SG_1 = String galvanometer to record thermocurrents. D = Dewar flask containing junction L_2. SG_2 = String galvanometer registering potential differences. R = Special electron tube with high insulation of grid. AS = Anode volts. HS = Heating current. (Buchthal & Lindhard).

containing liquid paraffin, the temperature of which could be kept constant within 0.01° C. In most of the experiments the temperature variations were recorded from one string of a double-string galvanometer, the other being used for recording the potentials. The arrangement was calibrated for each experiment. A difference of temperature of 1° C.,

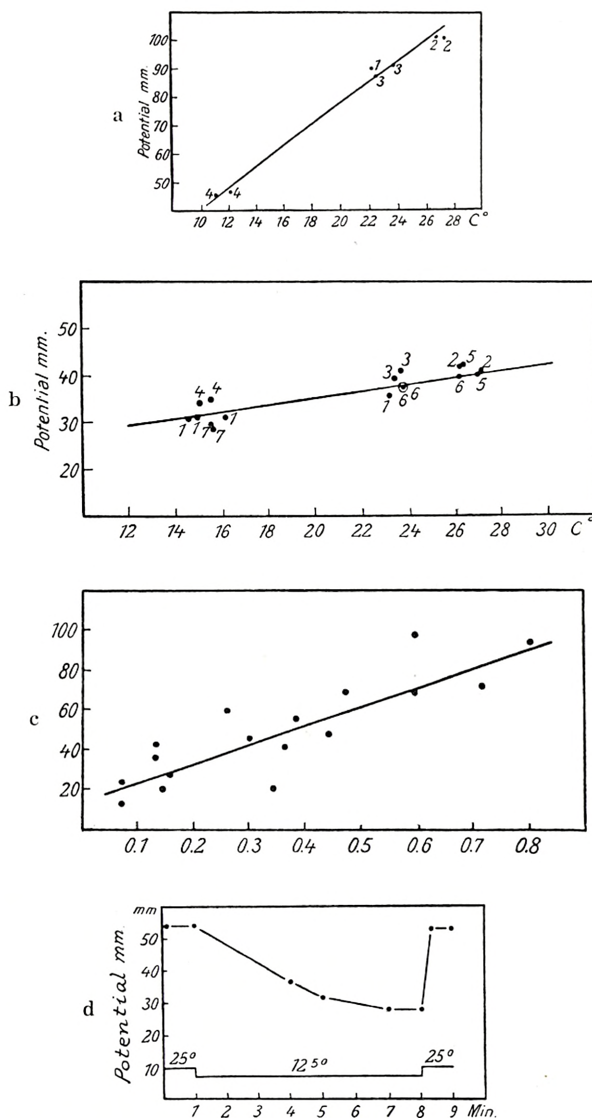

Fig. 62. a. and b. Fibre potential at different temperatures. Numbers indicate sequence of measurements. Ordinate: potential in mm. Abscissa: Temperature. c. Potential level and slope of potential/temperature curves. Ordinate: potential level. Abscissa: slope. d. Rate of adaption of potential with decrease and increase of temperature.
(BUCHTHAL & LINDHARD).

gave a deflection of 2—3 mm. on the string galvanometer. The whole of the experimental arrangement functioned satisfactorily in the tests performed; the greatest difficulty appeared to be that of obtaining absolute tightness between cover-glass and vulcanite capsule, but it was managed to secure this by leading the water through the capsule at a slight negative pressure. The slightest leakage, however, was shown at once by great disturbances of the potentials. The readings were not taken until both the thermo-element and the thermometer in the capsule had attained to constant values.

The result of this examination was that within the range examined the fibre potential rose and fell with the temperature (Figs. 62 a & b), provided this did not reach a level higher than 26—28° C. At temperatures as high as this, irreversible changes of the fibre usually occurred, so that only very rarely did the potential fall again with decreasing temperature. Otherwise these potential variations were reversible and reproduceable. It was, however, difficult to find a formula covering all the experimental results. Calculation of the temperature coefficient according to the formula

$$Q_{10} = \left(\frac{p_1}{p_2}\right)^{\frac{10}{t_1 - t_2}}$$

where p denotes the potential difference and t the temperature, gave as a mean figure 1.4 ± 0.03 with an average error for a single determination of 22 per cent. A systematic variation of Q_{10} with temperature could not be demonstrated. Calculation of b according to Bělehrádek's (1935) formula,

$$b = \frac{\log p_1 - \log p_2}{\log (t_1 - \alpha) - \log (t_2 - \alpha)}$$

where α is the difference between the "biological zero" and the zero of the thermometric scale, gave $b = 1.08 \pm 0.05$ with an average error of 45 per cent. Lastly, a comparison of the percental variation of temperature with the percental alteration of the potentials did not suggest any connection between the results. The difficulty of finding a general formula for the results of the experiments is probably due to the fact that these experiments cannot be considered comparable in simple terms, for the slopes of the potential-temperature curves were different for each experiment (Figs. 62 a & b) proportional to the level of the potential, a more abrupt temperature curve corresponding to a higher initial value of the potential level. Fig. 62 c shows the relation between the level of the potential and the value for the slope of the temperature curve. The cause of this interdependence is unknown.

The potential change on cooling was fairly slow, taking place in about 6 minutes, in the case of a fall in temperature from 25 to 12.5° C., whilst a subsequent increase to 25° C., resulted in increase of the potential to its former level in the course of a few seconds (Fig. 62 d). A similar phenomenon is seen in the variations of viscosity of hydrophilic colloids with temperature (Loeb 1922).

Finally it may be mentioned that the influence of temperature changes on both fibre-fibre and fibre-end-plate potentials, is much greater than if they were proportional to the absolute temperature, as Bernstein (1902) has found for injured preparations.

The resistance of the individual muscle fibre in situ as well as of the isolated fibre was measured by Buchthal (1934, 1935) in two different ways. Only experiments in which the isolated fibre was used will be discussed here,

since they at the present time afford the best information as to the resistance of the contractile elements.

In these experiments, fibres of the M. obl. abd. int. and limb muscles of the frog were used in situ and fibres of isolated muscles of frogs and lizards. For examination of fibres in situ the animal under urethane was placed on a paraffined glass-plate on the stage of the microscope. As potential source the fibre potential referred to above was used; non-polarizable micro-electrodes were employed for leading off. Electrodes were selected whose openings, as far as possible, were of equal size, and their resistance was determined before and after each experiment. The possible potential difference between the electrodes never exceeded 0.5 mV.

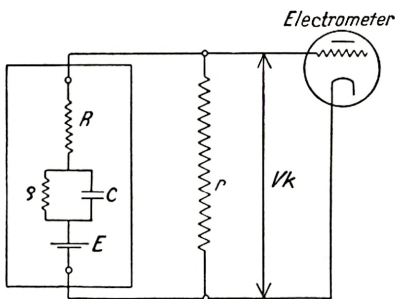

Fig. 63. Diagram of model. $E =$ voltage, $R =$ resistance and $C =$ capacity; $\varrho =$ shunt resistance to C. $r =$ input resistance of electrometer, the variation of which permits measurements of $R + \varrho$. (Buchthal).

The method of measurement consisted in an electrostatic determination of the voltage, E, which was then loaded with a resistance, r, of 40, 10 and 6 meg. ohms in turn, and the voltage V_k then measured (Fig. 63). When E, V_k and r are determined, the total resistance can be calculated according to the formula

$$R + \varrho = r \frac{E - V_k}{V_k}.$$

The resistance determined in this manner was independent of variations in the resting potential of the fibre. The latter was constant for long periods when the preparation

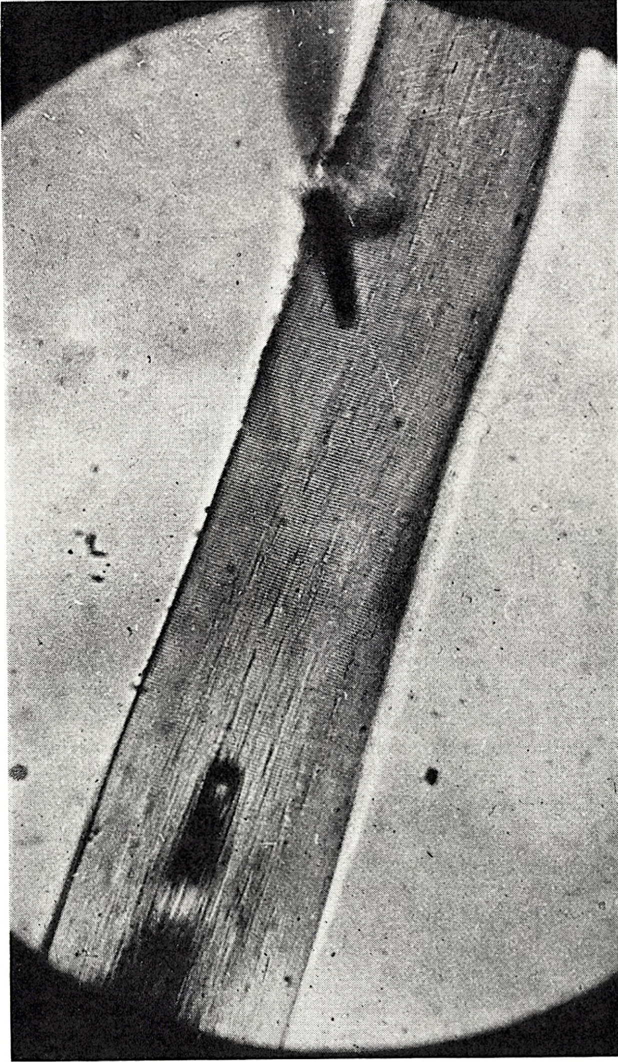

Fig. 64. Single frog's muscle fibre with micro-electrodes in position. (BUCHTHAL).

was covered with liquid paraffin. As the measurement took a short time only, the temperature could also be kept constant during the experiment. By means of control experiments

on dead muscles it was made certain that there was no significant resistance between preparation and electrodes. As the method requires both electrodes to be placed on the same fibre (Fig. 64), some measurements of fibre potentials with varying distances between the electrodes were made before each experiment; if these fibre potentials varied regularly with the distance between the electrodes, this was considered to indicate that the electrodes were on the same fibre. For the same reason comparatively thick fibres were usually selected for the experiment. As the resistance of fibres depends upon their thickness, the diameter of the fibre must be measured, and, as has previously been mentioned, this can only be done approximately on fibres in situ; accurate measurements can, however, be made on isolated fibres; Table XI, shows the results of a number of such measurements on living, resting muscle fibres.

As the principal result of the experiments BUCHTHAL found that the resistance of a resting fibre of about 70 μ diameter with a distance between the electrodes of 400 μ, was about 3 meg. ohm. For purposes of comparison it can be stated that a column of 0.6 per cent. NaCl solution of the same dimensions has a resistance of 100,000 ohm. The specific resistance of the fibre will thus be 3000 ohm/cm. against that of the salt solution of 109 ohm/cm.

Whilst the resistance of fibres of different frogs might vary by 20 to 30 per cent., it was fairly constant in different fibres of the same animal, when the thickness of the fibres was taken into consideration. Measurements on isolated fibres generally gave a somewhat higher result than on those in situ, which is probably due to the fact that the single paraffin-covered fibres are better isolated from their sur-

Table XI.

Preparation Nr.	Fibre Diameter in μ	Resistance of resting fibre in meg. Ω. Distance between electrodes, μ.		
		600—400	400—200	200—50
1	115	3.81	2.04	1.24
	110	—	2.27	—
	110	—	2.27	0.92
	80—82	—	2.26	—
	70	—	3.17	2.82
2	90	..	2.50	0.82
	90—95	..	2.84	—
3	120	..	1.24	—
	120	..	1.48	0.80
	120	..	1.25	2.95
	130	..	1.88	1.15
4		2.86	2.11	..
		3.20	2.12	..
	80—100	2.97	2.30	..
		3.34	—	..
		3.64	2.50	..
5	80	—	2.21	1.47
	75—80	—	1.77	1.67
	80	—	2.22	1.50
	100	2.12	1.64	1.42
6	120	..	2.10	1.85
	100—110	..	1.95	—
7	90	2.97	2.12	—
	95—100	2.50	2.85	—
	55	4.54	3.48	1.98
8	120	..	1.70	1.10
	90	..	2.00	1.84
9	90	2.65	—	..
	90	2.40	2.03	..
	80	—	2.05	..
	80	—	2.12	..
10	70	3.60	2.70	..
	70	—	2.90	..
	60—80	—	2.73	..
	60—80	—	2.70	..
	60—80	—	2.10	..

Table XI (continued).

Preparation Nr.	Fibre Diameter in μ	Resistance of resting fibre in meg. Ω. Distance between electrodes, μ.		
		600—400	400—200	200—50
11	90	3.43	3.04	. .
	100	3.51	—	. .
	100	3.14	—	. .
	60	4.75	—	. .
	60	4.81	4.43	. .
	65	4.63	4.00	. .
	65	4.90	—	. .
12	110	2.20	. .	1.58
	about 45	4.95	. .	—
13	90—115	. .	2.97	—
			3.02	2.65
			2.85	—
			2.72	—
			2.92	—
			2.22	—
14	70	5.22	—	. .
	70	—	3.42	. .
	70	—	4.30	. .

roundings than are fibres in contact with others and with capillaries.

The results arrived at by means of the method outlined

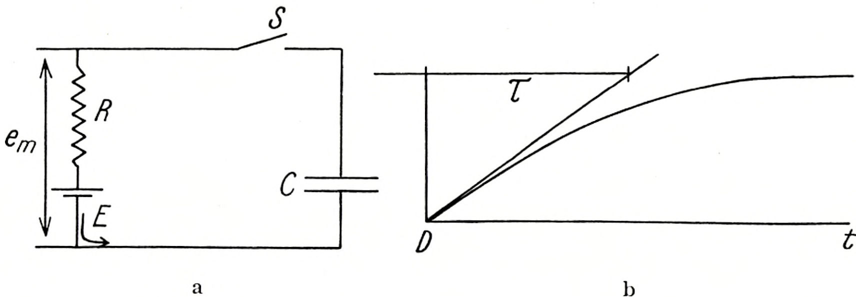

Fig. 65. (a.) Measurement of the resistance R by recording time course of e_m, after closure of S. (b). exponential eourse of e_m. Abscissa: time. Ordinate: potential difference. τ = time constant. (Buchthal).

above were controlled by experiments using another method. The resting potential of the fibre was again used as source of voltage, which from the point of view of measurement is an advantage because, if appreciable voltages are introduced from without, a new and incalculable source of error thus arises. The internal resistance was determined by recording the time-curve of the potential e_m (Fig. 65), a known capacity C being placed in parallel with the fibre. The condenser must be at zero voltage at the moment of connection. Then the required resistance, R, can be determined from the time constant, τ, and the capacity of the condenser, C, i. e. $R = \tau/C$. For details of the calculations reference may be made to the original paper. The reliability of the method was ascertained by a number of controls; otherwise the preparation of the tissue and the recording arrangements

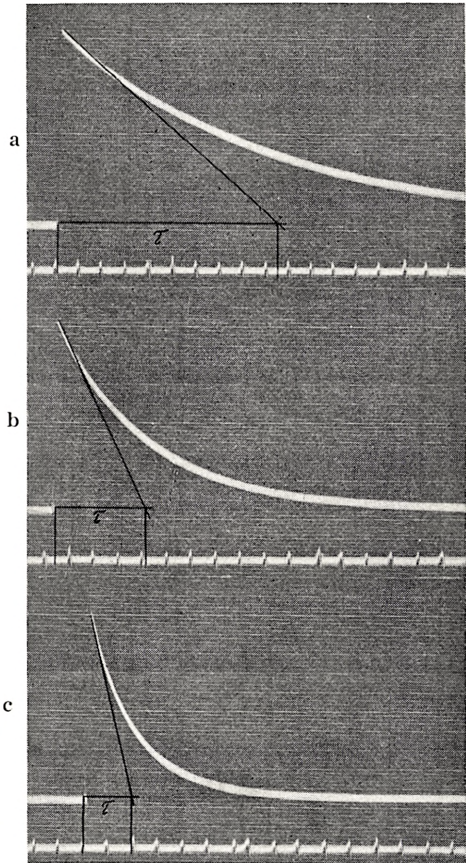

Fig. 66. Decrease of potential after shunting with different capacities. Time $^1/_5$ sec.

	Capacity	sec.	Resistance
a	0.5 μ F	1.9	3.8 meg. Ω.
b	0.2 μ F	0.24	3.7 meg. Ω.
c	0.1 μ F	0.38	3.8 meg. Ω.

(Buchthal).

were the same as in the series of experiments first reported. Fig. 66 is reproduced as an example of the measurements. As shown by Fig. 67, the charging of the condenser takes place according to a purely exponential curve, proving that appreciable disturbances as a consequence of polarization counter-forces do not occur.

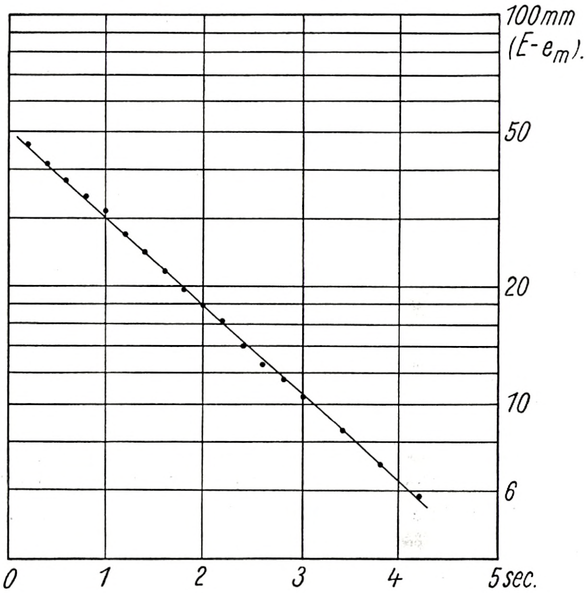

Fig. 67. Curve 66 a plotted on semilogarithmic paper. Ordinate: $E-e_m$. Abscissa: time in sec. (BUCHTHAL).

By means of the method first referred to, BUCHTHAL also examined the change of resistance when the fibre was stimulated. Though indirect stimulation of isolated fibres entails some experimental difficulties, it was still considered necessary to employ this procedure besides direct stimulation. The preparations employed were similar to those previously described. In these experiments the nerve was exposed just at its exit from the spinal canal, and then prepared according to the method of ADRIAN & BRONK (1928) so that stimulation

produced contraction of a few fibres in the field of vision; in two cases one fibre only responded. In order to safeguard as far as possible against external influences in the cases where more than one fibre reacted, the distance between the electrodes was made comparatively short, only 150—200 μ. High frequency currents were used for stimulation. The terms in the formulae stated above for calculation of the

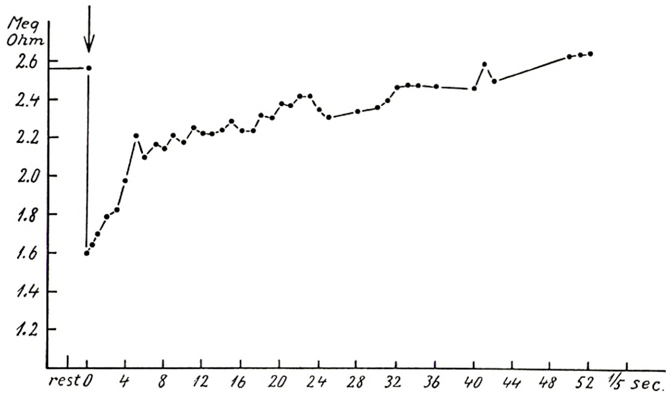

Fig. 68. Variation of the resistance of a single fibre after stimulation. Abscissa: time in 1/5 sec.; ↓ Stimulus. (Buchthal).

resistance must of course have been determined at the same time in relation to the moment of stimulation.

The results of these measurements show that immediately after a direct or indirect stimulus the resistance suddenly decreases by 30—50 per cent., resuming its resting value in 4—8 seconds (Fig. 68, Table XII). The recovery curve is of exponential form comprising two parts, the first having a rapid course, which then passes into the slower almost straight second part. In about one third of the cases examined the transition between the two parts is distinctly marked by a sudden increase of short duration in the resistance. These changes of resistance cannot be due to

Table XII.

Experiment Nr.	Resistance of resting fibre in meg. Ω	Resistance during contraction in meg. Ω	Time of recovery in $^1/_5$ sec.
1	6.6	3.6	6
2	2.3	1.8	13
3	1.5	0.6	16
4	2.55	1.6	50
5	4.6	2.9	13
6	2.3	1.95	8
7	2.1	1.7	13
8	1.7	0.6	6
9	3.6	2.2	12
10	1.9	0.6	—
11	4.0	2.6	6
12	3.6	2.6	incomplete recovery
13	2.2	0.9	6
14	3.5	3.7	6
15	1.9	1.1	12
16	5.7	5.1	5
17	2.3	1.8	5

changes in shape of the fibres, as the latter will only be able to change their thickness, and then not throughout their length. As demonstrated by LINDHARD & MÖLLER, the fibre when it contracts becomes thicker in one part and thinner in another. This, however, could result in an increased resistance in one region of the fibre, which has never been observed though measurements have been made in many different parts of these. It must also be borne in mind that possible mechanical changes after stimulation take a much more rapid course than do the changes of resistance, even if possible elastic after-effects are taken into consideration. Impedance measurements on the whole muscle have shown that the A.C. resistance of the muscle as a whole increases after stimulation (BOZLER & COLE (1935) DUBUISSON (1937)). These experiments, however, are not directly comparable to those referred to above, firstly because they

were made on the whole muscle, and secondly because the resistance measurements were made transversely and not longitudinally as in the present experiments. The resistance changes found in the single fibre may have their origin in permeability changes, in variation of the number of ions or finally in an altered distribution in the conductive and

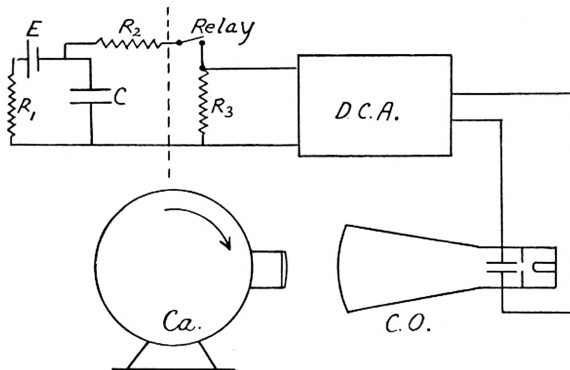

Fig. 69. Device for measuring electrostatic capacity.

E	= voltage	model.
R_1	= internal resistance of E	
R_2	= resistance in series	
R_3	= input resistance.	
D. C. A.	= direct current amplifier.	
CO	= cathode ray oscillograph.	
ca	= camera.	(BUCHTHAL & NIELSEN).

non-conductive elements of the fibre. For the time being it is impossible to assess the relative importance of these factors.

The capacity of the muscle fibre has yet to be discussed. It was determined by BUCHTHAL and NIELSEN, who tried to reduce the complicated conditions of the fibre to a relatively simple model. They assumed the presence of a condenser, which was charged by the voltage E (fibre potential) through a resistance R_1 (see Fig. 69). Besides reckoning with

this internal resistance, there is also a leading off resistance, R_2. For calculation of the capacity use was made of the time variation of the voltage E, when a resistance, R_3, is inserted in parallel with the condenser. Fig. 70 shows the course of the curve. When R_3 is inserted the voltage suddenly increases, falling again according to an exponential curve to its previous value. The connection with R_3 was effected by means of the relay referred to above, and the change of the potential was registered through a D.C. amplifier by means of a cathode ray oscillograph. It appeared that the course of the curve in experiments on living muscles was the same as in the model experiments, whilst dead muscles, or an artificially produced potential difference between the two electrodes gave curves that were deflected almost at right angles. We may suppose, therefore, that the course of the curve in experiments with living fibres implies that they possess accumulated electrostatic energy, which is related in some way to the normal structure of the fibre.

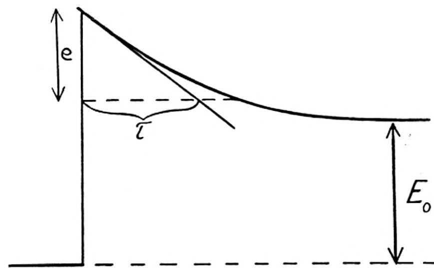

Fig 70. Variation of fibre potential after shunting with R_3 (see text). (BUCHTHAL & NIELSEN).

Calculation of the capacity is made according to the formulae below, the quantities τ, E_0, E, e, and R_3 being known, or measured from the curves, R_1, R_2, and C being unknown.

$$E_0 = \frac{E \cdot R_3}{R_1 + R_2 + R_3}$$

$$E_0 + e = \frac{E \cdot R_3}{R_2 + R_3}$$

$$\tau = \frac{R_1 (R_2 + R_3)}{R_1 + R_2 + R_3} \cdot C.$$

When these equations are solved we arrive at the following results:—

$$R_1 = \frac{e}{E_0} \cdot \frac{E}{E_0 + e} \cdot R_3$$

$$R_2 = R_3 \left(\frac{E}{E_0 + e} - 1 \right)$$

$$C = \frac{\tau (R_1 + R_2 + R_3)}{R_1 (R_2 + R_3)}.$$

E is stated in mV, R in meg. ohm, C in μF, and τ in seconds.

Experiments on muscles of different animals give some variation in the results, all of which, however, are of the same order of magnitude. The result of a single experiment will be stated here; with $R_3 = 6$ meg. ohm it was

$$R_1 = 0.2 \text{ meg. ohm}, \; R_2 = 1.3 \text{ meg. ohm}, \; \tau = \frac{3.5}{50} \text{ sec. and}$$
$$C = 0.36 \;\mu\text{F}/100\;\mu$$

The measurements of potential and capacity referred to above make it possible to form a rough idea of the size of the electrostatic forces in the muscle fibre. It must, however, be realized that it cannot be more than an estimate, because it is not yet possible to determine the potential within the sarcolemma of the undamaged fibre under physiological conditions. The potentials hitherto measured are lower than the physiological potentials.

The electrostatic potential energy of the fibre can be determined according to the formula

$$\text{Energy} = \frac{1}{2}\, 10^{-5} \cdot C\mu\text{F} \cdot E^2\text{mV} \;\; \text{Erg}/100\ \mu.$$

If the capacity calculated from the above experiment and the value stated above (p. 154) for the potentials measured intralemmally are introduced into this equation we find

$$\text{The energy} = \frac{1}{2}\, 10^{-5} \cdot 0.36 \cdot 45^2 \cdot 10^2 = 0.37 \;\; \text{Erg/cm.}$$

Similar difficulties and uncertainties are encountered when it is desired to determine how great a mechanical tension a muscle fibre is able to develop at its equilibrium length. In reality there are very few suitable determinations of tension. Exact determination of the tension of a single isolated fibre in the case of a so-called single contraction has not yet been made. As mentioned previously, ASMUSSEN has determined the tension of small bundles during a tetanus of short duration; but measurements of the thickness of the fibres, were not made. This would have been desirable because the tensions were measured on fibres of limb-muscles, whilst potentials and capacity were measured on abdominal muscles. On the existing basis, therefore, we cannot get further than to an approximate determination of the order of magnitude of mechanical energy. In an experiment with a bundle of 12 fibres ASMUSSEN states the tension at equilibrium length to be about 60 mg. which, when incorporated in the formula for the potential mechanical energy, $^1/_5$ Tl (p. 68), gives about 1 Erg/cm., which is about 3 times the electrical energy calculated above. Both quantities are, however, based on factors so many of which are at present uncertain, that in view of the numerous, in part uncontrollable sources of error, we cannot expect more at present; on the other hand it appears to be justifi-

able, all things considered, to maintain that the existing results confirm rather than invalidate the hypothesis of contraction here advanced. Further investigations will comprise determination of the magnitude of the potential difference in the undamaged fibre and measurement of the mechanical tension of individual fibres under physiological conditions.

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References.

Adrian, E. D. Arch. neerl. Physiol. 1922. **7.** 330.
— J. Physiol. 1925. **60.** 301.
— Ergebn. Physiol. 1933. **35.** 744.
— and Bronck, D. W. J. Physiol. 1928. **66.** 81.
— and Gelfan, S. J. Physiol. 1933. **78.** 271.
Agduhr, E. Anat. Anz. 1916. **49.** 1.
— Anat. Anz. 1919. **52.** 273.
Apathy, St. Proc. VII. Int. Zool. Kongr. Boston 1907. p. 177.
Asmussen, E. III. Nordisch. Kongr. f. Physiol. Skand. Arch. 1931. **63.** 1.
— Pflügers Arch. 1932. **230.** 263.
— Skand. Arch. 1933. **65.** 261.
— Skeletmuskelfibrens mekaniske Reaktion (Disputats). Copenhagen 1934.
— Skand. Arch. 1934. **70.** 233.
— Skand. Arch. 1935. **72.** 51.
— Skand. Arch. 1936. **74.** 129.
— 19. Nordisk Naturforskarmøtet. Helsingfors 1936.
— and Lindhard, J. Det kgl. Danske Vidensk. Selsk. Biol. Medd. XI. 1933.
— — Skand. Arch. 1935. **72.** 240.
Astbury, W. T. Nature 1936. **137.** 803.
— and Dickinson, S. Nature 1935. **135.** 95.
— — Nature 1935. **135.** 765.
— — Nature 1936. **137.** 909.
Aurell, G. and Wohlfahrt, G. Jahrb. f. Morphol. und mikr. Anatomie. II Abt. 1936. **40.** 402.

Babuchin, A. Ztbl. Med. Wiss. 1870. 241.
Bardeen, C. R. Anat. Anz. 1903. **23.** 241.
Bělehrádek, I. Temperature and Living Matter. Monogr. Protoplasma. Berlin 1935.
Bernstein, I. Pflügers Arch. 1902. **92.** 521.

Bernstein, I. and v. Tschermak, A. Pflügers Arch. 1906. **112.** 439.
Bethe, A. and Happel, P. Pflügers Arch. 1923. **201.** 157.
Beutner, R. Entstehung elektr. Ströme im lebenden Gewebe. Stuttgart 1920.
Biedermann, W. Erg. Biol. 1927. **2.** 433.
Blix, M. Skand. Arch. 1891. **3.** 295.
— Skand. Arch. 1895. **5.** 150 and 173.
Boeke, I. Z. mikr. anat. Forsch. 1926. **7.** 95.
— Z. mikr. anat. Forsch. 1927. **8.** 561.
— Z. mikr. anat. Forsch. 1933. **34.** 330.
Bozler, E. Amer. J. Physiol. 1934. **14.** 109.
— J. comp. a. cell. Physiol. 1935. **6.** 217.
— Fortschr. Zool. 1937. **1.** 334.
Brinch-Eliassen, I. Skeletmusklens Følsomhed etc. (Disputats 1925) (English summary).
Briscoe, Grace. Quart. J. exp. Physiol. 1928. **19.** 1.
Brown, D. I. S. and Sichel, F. I. M. Science 1930. **72.** 17.
— — J. cell. a. comp. Physiol. 1936. **8.** 315.
Brown, G. L. J. Physiol. 1937. **89.** 220.
— Dale, H. H. and Feldberg, W. J. Physiol. 1936. **87.** 394.
Buchthal, F. Skand. Arch. 1934. **68.** 187.
— Skand. Arch. 1934. **70.** 199.
— Skand. Arch. 1935. **72.** 63.
— Skand. Arch. 1937. **77.** 20.
— Skand. Arch. 1937. **77.** 158.
— Arch. f. exp. Zellforsch. 1937. **19.** 177.
— J. Physiol. 1937. **90.** 37.
— Proc. Roy. Soc. B. 1937. **123.** 404.
— and Knappeis, G. G. Zeitschr. wiss. Mikrosk. 1935. **52.** 37.
— — Zeitschr. f. wiss. Photogr. 1936. **35.** 177.
— — and Lindhard, J. Skand. Arch. 1936. **73.** 163.
— — — Nachtrag. Skand. Arch. 1936. **73.** 163.
— — Skand. Arch. 1938. **78.** 97.
— and Lindhard, J. Skand. Arch. 1934. **70.** 227.
— — Skand. Arch. 1935. **72.** 35.
— — Skand. Arch. 1936. **74.** 223.
— — Sympos, ded. to Prof. Beritoff. Tiflis 1936. p. 111.
— — Skand. Arch. 1937. **77.** 224.
— — J. Physiol. 1937. **90.** 2.
— and Nielsen, I. O. Skand. Arch. 1936. **74.** 202.

BUCHTHAL, F. and PERSSON, CHR. L. J. Scient. Inst. 1936. **13.** 20.
— — Compt. rend. XII. Congr. Int. Zoolog. 1935. 821.
— and PÉTERFI, T. Pflügers Arch. 1934. **234.** 527.
— — Protoplasma 1937. **27.** 473.
BÖHM, G. Med. Kolloidlehre. Abt. II. Dresden 1933. **10.** 609.
— and WEBER, H. H. Kolloid Zeitschr. 1932. **61.** 269.

CARVALLO, I. and WEISS, G. Pflügers Arch. 1899. **75.** 591.
McKEEN CATTELL. J. Physiol. 1932. **75.** 264.
CIACCIO, G. Boll. Soc. Ital. Biol. sper. 1936. **11.** 221 a. 1936. **11.** 798.
COLE, K. S. and CURTIS, H. J. Cold spring Harbor Sympos. Quant. Biol. 1936. **4.**

DALE, H. H. Bull. New York Acad. Medicine 1937. **13.** 379. Harvey Lecture.
D'ANCONA, U. Protoplasma 1930. **10.** 177.
— Boll. Soc. Ital. Biol. sper. 1931. **6.** 1.
— Boll. Soc. Ital. Biol. sper. 1935. **10.** 360.
DRESER, H. Arch. f. exp. Pathol. 1890. **27.** 50.
DUBUISSON, M. Les Ionogrammes de la Contraction musculaire. Paris 1935.
— J. Physiol. 1937. **89.** 132.

ECCLES, J. C. Ergebn. Physiol. 1936. **38.** 383.
EISENBERGER, J. P. Amer. J. Physiol. 1918. **45.** 44.
ENGELMANN, TH. W. Pflügers Arch. 1873. **7.** 33.
— Pflügers Arch. 1873. **7.** 155.
— Pflügers Arch. 1878. **18.** 3.
ERNST, E. and KOCZKÁS, I. Z. Biol. 1935. **96.** 185.
— — Z. Biol. 1935. **96.** 201.
ETTISCH, G. and PÉTERFI, T. Pflügers Arch. 1925. **208.** 454.
EWART, I. C. Philos. Transact. 1888. **179.** 379 a. 539.
— Philos. Transact. 1892. **183.** 79.

FICK, A. Die Muskeltätigkeit. Leipzig. 1882.
— Mechan. Arbeit und Wärmeentwicklung bei der Muskeltätigkeit. Leipzig. 1882.
— Pflügers Arch. 1892. **51.** 541.
FISCHER, E. Amer. J. Physiol. 1931. **96.** 78.
— J. cell. a. comp. Physiol. 1935. **5.** 441.

FISCHER, E. Amer. J. Physiol. 1935. **113.** 42.
— Cold. Spring Harbor Sympos. Quant. Biol. 1936. **4.** 214.
FISCHL, E. and KAHN, R. H. Pflügers Arch. 1928. **219.** 33.
FUJI, K. J. coll. Scient. Univ. Tokyo. 1914. **27.** 1.

GAD, I. Arch. f. Physiol. 1879. 250.
GARNER, W. E. Proc. Roy. Soc. B. 1925. **99.** 40.
GASSER, H. S. and HILL, A. V. Proc. Roy. Soc. B. 1924. **46.** 398.
GELFAN, S. Amer. J. Physiol. 1930. **93.** 1.
— Amer. J. Physiol. 1931. **96.** 16.
— J. Physiol. 1933. **80.** 283.
— and BISHOP, G. G. H. Amer. Journ. Physiol. 1932. **101.** 678.
— — Amer. J. Physiol. 1933. **103.** 237.
— and GERARD, R. W. Amer. J. Physiol. 1930. **95.** 412.

HANSEN, F. C. C. Personal communication.
HAPPEL, P. Pflügers Arch. 1926. **213.** 336.
HEIDENHAIN, M. Pflügers Arch. 1869. **2.** 423.
— Plasma und Zelle. Jena 1911.
HEIDERMANNS, C. Zool. Jahrbuch 1935. **55.** 64.
HENRIQUES, V. and LINDHARD, J. Pflügers Arch. 1920. **183.** 1.
— — Pflügers Arch. 1923. **200.** 11.
HERMANN, L. Untersuchungen zur Physiologie der Muskeln und der Nerven. 2. Hälfte, 1867. 3. H. 1868.
— Pflügers Arch. 1870. **3.** 15.
— Pflügers Arch. 1871. **4.** 149.
— Pflügers Arch. 1877. **15.** 227.
HILL, A. V. J. Physiol. 1911. **42.** 1.
— J. Physiol. 1913. **46.** 435.
— J. Physiol. 1926. **61.** 499.
— Muscular Activity. Baltimore 1926.
— Proc. Roy. Soc. B. 1930. **107.** 115.
— Proc. Roy. Soc. B. 1931. **109.** 267.
HINSEY, I. C. Physiol. Rev. 1934. **14.** 514.
HOLTZ, B. Pflügers Arch. 1932. **230.** 246.
HÜRTHLE, K. Pflügers Arch. 1909. **126.** 1.
— Pflügers Arch. 1930. **233.** 685.
— Pflügers Arch. 1931. **227.** 585.
— Pflügers Arch. 1931. **227.** 610.
— Pflügers Arch. 1931. **227.** 637.

Häggquist, G. Anat. Anz. 1920. **3.** 273.
— Zeitschr. mikr. anat. Forsch. 1925. **4.** 605.
— Hdb. d. mikr. Anat. 1931. **2.** Teil 3. 105.
Höfer, P. Z. Biol. 1933. **94.** 247.

Jordan, H. E. Physiol. Rev. 1933. **13.** 301.
— Amer. J. anat. 1934. **117.** 55.

Kato, G. The Microphysiol. of Nerve. Tokyo 1934.
Krause, W. Henle & Pfeuffer 1868. **33.** 265.
— Z. Biol. 1869. **5.** 411.
Krogh, A. Biochem. Zeitschr. 1914. **62.** 266.
Krüger, R. Pflügers Arch. 1928. **219.** 66.
Kühne, W. Arch. Anat. Physiol. 1860. 477.
— Unters. aus d. Physiol. Inst. Heidelberg 1882. II. 187.
— Z. Biol. 1887. **23.** 1.

Langer, G. Monit. Zool. Ital. 1937. **47.** 325.
Lindhard, J. Det Kgl. Danske Vidensk. Selsk. Biol. Medd. 1924. IV.
— Physiol. Papers ded. to Prof. Aug. Krogh. Copenhagen 1926. p. 188.
— Ergebn. Physiol. 1931. **33.** 337.
— Skand. Arch. 1932. **64.** 299.
— Skand. Arch. 1934. **69.** 59.
— and Møller, J. P. J. Physiol. 1926. **61.** 73.
— — Skand. Arch. 1928. **54.** 41.
— — Det Kgl. Danske Vidensk. Selsk. Biol. Medd. 1930. VIII.
Loeb, J. Proteins and the Theory of Colloidal Behaviour. New York and London 1922.
Lubosch, W. Morphol. Jahrbuch 1937. **80.** 89.
Lucas, Keith. J. Physiol. 1905. **33.** 125.
— J. Physiol. 1907. **36.** 113.
— J. Physiol. 1909. **38.** 115.
— J. Physiol. 1909—1910. **39.** 207.

Mayeda, R. Zeitschr. Biol. 1890. **27.** 119.
Meyer, K. H. Biochem. Zeitschr. 1929. **208.** 211.
— Biochem. Zeitschr. 1929. **214.** 253.
— and Mark, H. Der Aufbau der hochpolymeren, organischen Naturstoffe. Leipzig 1930.

Meyer, K. H. and Ferri, C. Pflügers Arch. 1936. **238.** 78.
— and Picken, L. E. R. Proc. Roy. Soc. B. 1937. **124.** 29.
Meyerhof, O. Die chem. Vorgänge im Muskel. Berlin 1930.
— and Hartmann, H. Naturwissenschaft 1933. **21.** 661.
— — Pflügers Arch. 1934. **234.** 722.
— and Möhle, W. Biochem. Zeitschr. 1933. **260.** 454.
— — Biochem. Zeitschr. 1933. **260.** 469.
— — Biochem. Zeitschr. 1933. **261.** 252.
— — Pflügers Arch. 1935. **236.** 533.
— — Biochem. Zeitschr. 1936. **284.** 1.
Monnier, A. M. L'excitation éléctrique des tissue. Paris 1934.
— Cold Spring Harbor Sympos. 1936. **4.** 111.
Muralt, A. v. Pflügers Arch. 1932. **230.** 299.
— Kolloid-Zeitschr. 1933. **63.** 228.
— Ergebn. d. Physiol. 1935. **34.** 406.
— and Edsall, J. T. J. biol. Chem. 1930. **89.** 315.
— — J. biol. chem. 1930. **89.** 351.
Möllendorff, W. v. Oppenheimers Hdb. d. Biochem. 1925. **2.** 273.

Nagamitû, G. Okkoyama-Igakkai-Zasshi 1931. **34.** 2879. Autoref. Ber. Physiol. 1932. **65.** 215.
Nagel, A. Z. Zellf. 1935. **22.** 694.
Nicolai, L. Pflügers Arch. 1936. **237.** 399.
Nielsen, I. O. Zeitschr. f. Physik 1937. **107.** 192.
Noll, D. and Weber, H. H. Pflügers Arch. 1934. **235.** 234.

Péterfi, T. Arch. mikrosk. Anat. 1913. **83.** 1.
— Biol. Zentralbl. 1935. **55.** 86.
— Fortschr. d. Zoolog. 1937. **1.** 19.
— Fortschr. d. Zoolog. 1937. **2.** 17.
Petersen, H. Histol. und mikrosk. Anat. München 1935. p. 844.
Piper, H. Elektrophysiol. d. mensch. Muskeln. Berlin 1912.
Pischinger, A. Pflügers Arch. 1927. **217.** 205.
Policard, A. and Okkels, G. Abd. Handb. d. biol. Arbeitsmeth. Abt. V. Teil II. 1931. p. 1816.
Pratt, F. H. and Eisenberger, J. P. Amer. Journ. 1919. **49.** 1.

Riesser, O. Erg. Physiol. 1936. **38.** 133.
— and Miura, R. Pflügers Arch. 1933. **232.** 513.

Riesser, O. and Schneider, W. Pflügers Arch. 1929. **221.** 713.
Ritchie, A. D. J. Physiol. 1933. **78.** 322.
Rosemann, H. H. Zeitschr. Biol. 1936. **97.** 55.
Rushton, W. A. H. J. Physiol. 1932. **75.** 445.
Ryden, Å. and Wohlfart, G. Zeitschr. mikr., anat. Forsch. 1932. **29.** 605.

Sachs, C. Arch. Anat. u. Physiol. 1874. 57.
Sandow, A. J. cell. and comp. Physiol. 1936. **9.** 37.
— J. cell. and comp. Physiol. 1936. **9.** 55.
Schiefferdecker, P. Ref. Schwalbes Jahresber. 1902.
Schimert, J. Z. mikr. anat. Forsch. 1934. **36.** 215.
— Z. mikr. anat. Forsch. 1934. **37.** 581.
Schmidt, W. J. Die Bausteine des Tierkörpers im polarisierten Licht. Bonn. 1924.
— Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma. Protoplasma Monogr. 1937.
Schütz, E. Ergebn. Physiol. 1936. **38.** 493.
Schäfer, H. Pflügers Arch. 1936. **237.** 329.
— and Göpfert, H. Pflügers Arch. 1937. **238.** 684.
Sichel, F. J. M. J. cell. a. comp. Physiol. 1934. **5.** 21.
Spiegel, E. A. Der Tonus der Skelettmuskulatur. Berlin 1927.
Steiman, S. E. Amer. J. Physiol. 1937. **118.** 492.
Steinhausen, W. Abd. Handb. biol. Arbeitsmeth. 1928. **5A.** 575.
Studnička. F. K. Z. f. Zellforsch. mikrosk. Anat. 1937. **26.** 36.
Studnitz, G. v. Z. f. vergl. Physiol. 1934. **21.** 440.
— Z. f. Zellforsch. u. mikr. Anat. 1935. **23.** 1.
— Z. f. Zellforsch. u. mikr. Anat. 1936. **25.** 373.
Stübel, H. Pflügers Arch. 1920. **180.** 209.
Sulzer, R. Z. Biol. 1930. **90.** 13.

Tiegs, O. W. Trans. Roy. Soc. S. Australia 1922—1923.
Tschirjew, S. Arch. Physiol. 1913. 414.

Wacholder, K. Handb. d. Neurolog. Vol. 2. 1. Berlin 1937.
Weber, E. Wagners Handwörterb. Physiol. Braunschweig 1846. Teil II.
Weber, H. H. Ergebn. Physiol. 1934. **36.** 109.
— Pflügers Arch. 1934. **235.** 205.

Wiener, O. Ber. d. Kgl. Sächs. Akad. Wiss. Math. Phys. Klasse. 1909. **61.** 113.
Wilkinson, H. J. Med. Journ. Australia. 1929. **16.** 768.
— J. comp. Neurol. 1934. **59.** 221.
Wohlfart, G. Anat. Anz. 1932. **74.** 228.
— Acta psychiatr. et neurolog. 1937. Suppl. XII.
Wöhlisch, E. and Clamann, H. G. Z. Biol. 1931. **91.** 399.

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