ON THE ULTIMATE STRUCTURE OF THE STRIPED MUSCLE FIBRE DISCERNIBLE WITH THE MICROSCOPE

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The light source, employed for this research, is supplied by an 8 V—6 A microprojection lamp (Philips). The rays, emitted by this wire-lamp are collected by means of a large lens and the bundle projected in the direction of the mirror of the microscope. A piece of groundglass, placed in the path of the bundle, diffuses the light. The plane mirror of the microscope reflects the central beam of the diffused light in the direction of the axis of the optical system. This beam, passing the aperture of the condensor diaphragm, uniformly fills up the plane of the aperture with light. The rays emerging from this uniformly illuminated plane actually constitute the primary light source. It is to this source that we shall repeatedly have to refer. The radiation, emanating from this source, is incoherent as it is evident that no permanent phase relations can exist between the rays of the diffused light.

The microscope is employed as an image-forming instrument and as a kind of interferometer. The image-forming constellation is characterized by the use of objective-lenses with a large numerical aperture, a widely opened condensor diaphragm and a strong condensor with a num. aperture between 1.20 and 1.40. By means of this condensor the rays emerging from the primary source are collected into a conical beam. The angle between an extreme ray and the central ray of the beam (α) forms the divergence angle of the illuminating bundle. The angle made by the central ray with

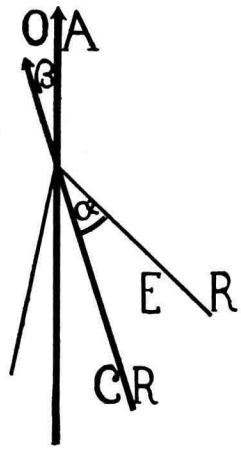

Fig. 1.
OA: optical axis; CR: central ray; ER: extreme ray;
α: divergence angle of incident beam; β: angle of incidence.

the optical axis (β) is called the angle of incidence. Fig. 1. By these two angles the mode of the illumination of the object plane is optically determined. A widely opened condensor diaphragm renders it possible to utilise fully the num. aperture of the condensor. The objectives made use of are a $^1/_{12}''$ and a $^1/_{16}''$ oil immersion lens of Leitz, with a num. aperture of 1.32 and a dry apochromatic lens of Reichert with a num. aperture of 0.95. These lenses, combined with a weak eye-piece, yield the following linear magnifications upon the photografic plate: the $^1/_{16}''$ lens of 500 times, the $^1/_{12}''$ lens of 400 times and the apochromate of 250 times. These enlargements represent the optima attainable with these objectives. Higher magnifications by using stronger eyepieces are merely illustrative.

Green and violet light has been chiefly used in the course of this research. Green light is made by means of a glass filter. This filter, however, allows broad bands of the spectrum to pass on either side of the green, particularly when the intensity of the source becomes very high. A solution of kaliumbichromate, placed before the filter, extinguishes the blue part of the spectrum that passes the filter. A solution of didymnitrate standing between the chromate filter and the mirror of the microscope, absorbs the yellow and orange rays and converts them into a green radiation. Red light of rather low intensity passes this combination of filters, however. The wave-length of the green rays lies between 570 and 535 millimicra. The maximum intensity of this band is situated in the neighbourhood of 555 millimicra. Rays of this wave-length are chosen for observation, because the optimal sensitiveness of the eye to light falls within this part of the spectrum. By working in a dark room the sensitiveness of the eye may be increased to slight gradations or to trifling differences in the brightness of the microscopic image. Violet light was obtained bij means of a selective glass filter. This filter, however, diminishes very markedly the intensity of the transmitted light. The wave-length of the rays passing through this filter lies between 465 and 425 millimicra, the maximum intensity of the band in the neighbourhood of 445 millimicra. This filter has also a second, much weaker, maximum of permeability in the neighbourhood of 760 millimicra. These rays, however, were no hindrance since their intensity is low and their wave-length too large to disturb appreciably the image formed by the violet rays. Violet light has been used for making photograms of structures imperfectly, or not at all, resolved by means of white light. Moreover, monochromatic light has the adventage of avoiding dispersion.

A microscopic object may be conceived as made up of discrete points (1). These points, if belonging to a flat object situated in the object plane of the microscope, can be made selfluminous by illuminating the object by means of a widely opened incident beam. An object point rendered perfectly selfluminous in this way emits a spherical wave-surface. The wave-front emanating from this surface, when it reaches the pupil of the objective lens, is diffracted at the circular margin of this aperture. The figure resulting from the diffracted waves, appears on the image-plane of the microscope as a small light-disk (Airy disk), surrounded by alternating dark and light rings. The brightness of the rings, which is much less than that of the disk, rapidly decreases towards the periphery of the diffraction-figure. Consequently the rings are generally not perceived, or if perceived, may be deliberately neglected. The light emerging from a disk is coherent because it is derived from a self-luminous point acting as a point source. It follows from the preceding that a microscopic image of an object rendered selfluminous is a point-for-point picture, and that the brightness of the picture is simply a result of the summation of the intensities of the overlapping diffraction disks.

It often proves to be difficult, if not impossible, to make the object points selfluminous and meantimes to optically independent sources of radiation when the points lie close together (2). In that case phase relations, resulting from the interdependence of the sources, may give rise to interferences superposed upon the image (3). This difficulty is encountered particularly when the object appears to have a regular multilayer structure the characteristic dimensions of which are commensurable with the wave-length of the visible light. Interferences superposed upon the image may then unexpectedly falsify the microscopic image.

An object point ceases to become perfectly selfluminous when the divergence angle of the illuminating beam is diminished by reducing the aperture of the condensor diaphragm. In this case the rays incident on the object are partly deflected bij the object points. The deflection becomes sharper, and the luminosity of the points less, when the aperture of the diaphragm is more and morer reduced. The deflected rays may give rise to interferences even at distances between the points greater than at what it is commonly believed interference can still occur. This may happen particularly when the incidence of the illuminating beam deviates but slightly from the normal to the object plane. These interferences, arising in the object-space of the microscope, superposed upon a fading image often render it impossible to recognise the real image simply by observation. A rough approximation, made for light of the middle band of the visible spectrum and for objective-lenses with a large num. aperture, indicates that the image dominates in these complex images when the divergence of the incident beam lies between 60° and 20°; when the angle lies between 20° and 10° the image can no longer be distinguished in the picture; when the angle is less then 10° the interference pattern becomes entirely preponderant (4).

Two disks, forming the image of a selfluminous double-point situated in the object plane of the microscope, may still be seen as separate parts of the image, if they just touch each other. The shortest distance between the two points $\triangle_{min}$ at which the disks are still represented in the image plane as separated, is given by the equation

$$\triangle_{min} = 1.22 \frac{\lambda_0}{A_{obj}}$$

In this formula λ_0 means the wave-length of the incident rays in air and A_{obj} the num. aperture of the immersion lens (1.32), supposing that the entire available aperture of the lens is utilized. This equation yiels numerical results in agreement with my measurements.

TABLE

	λ_0 in millimicra	$\triangle_{min}$ in micra
Red	640	0.60
Green	555	0.51
Blue	465	0.43
Violet	445	0.41

It may be deduced from these figures that the divergence of the light emitted by a selfluminous point of an object soaked in water, will have to be at least 80° if a strong immersion objective is used of which the entrance pupil can take up a beam with a divergence of 60° (num. apert. 1.32). Hence it is necessary, if using an oil immersion objective, that the points of the object become very nearly perfectly selfluminous in order to utilize the whole resolving power of the lens. It is for this reason that the condensor diaphragm must be wide open. This arrangement often involves the disadvantage that through scattering of incident rays the true structural dimensions of the object appears to be altered in the image.

A microscopic image has a noticeable dimension in the axial direction

of the optical system. This results partly from the imperfection of the lenses, partly it ensues from the wave properties of the light. In consequence of this dimension the image plane is not so sharply defined as the object plane. Small variations in the focussing of the microscope are therefore possible without deteriorating, or perceptibly changing the image. This will sometimes enable an observer, often unconsciously, to superpose an interference pattern, called forth by the structure of the object, upon the image of the object. This pattern sometimes may accentuate certain details of the image which are believed to be essential. Several traditional pictures have originated in this way.

The interferential arrangement forms a strict application of Abbe's spectrum theory of microscopic vision (5). It is characterized by the appliance of monochromatic light, a narrow illuminating beam and a central darkfield lens with an adjustable exit diaphragm. A narrow beam has been produced by projecting the image of the incandescent wire of the lamp upon the plane of the aperture in the condensor diaphragm. This aperture amounts to about 1.5 mm. The rays passing this narrow opening, are collected by a weak condensor into a beam with a small divergence angle. This beam passes a second fixed diaphragm situated between the condensor and the stage of the microscope. The aperture of this diaphragm amounts to 1 mm and is accurately centred. The edge of the aperture is made as smooth as possible in order to avoid irregular interferences near the margin of the aperture. The axial beam which this second diaphragm allows to pass, has a divergence angle equal to or less than 3°. The rays composing this beam are coherent, since they can be traced back to a very small spot of the radiating surface of the wire. For that reason the beam may be considered as originating in a point source, or as a beam virtually emitted by a source at infinite distance. A $^1/_{12}''$ oil immersion objective of Leitz has been transformed to a central darkfield lens by means of a minute opaque shield deposited upon the curved surface of the frontlens of the objective (6, 7). This shield screens off the central bundle (spectrum of zero order) of the diffracted incident beam, the beam being diffracted on its way through the object. The adjustable exit diaphragm of the objective-lens renders it possible to restrict the number of the diffraction spectra co-operating in the formation of the interference figure.

The interferential method has proved to be useful in making perceptible periodic structures which cannot be rendered visible, or very imperfectly so, by means of the image-forming constellation. Periodic structures occurring in living nature, are generally made up of ultimate elements appearing in a microscopic image as thread-like fibrils or as pointrows. A regular repetition of such elements in two or three dimensions constitutes a grating. An element together with its adjoining interstice forms the

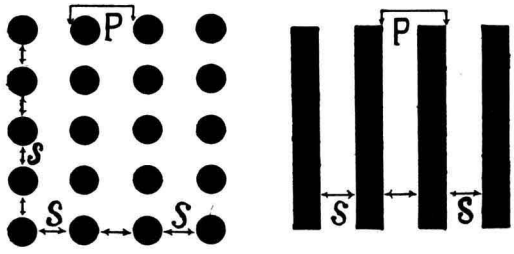

Fig. 2.
P: period of grating; S: spacing.

period (P) of the grating, the distance between two succeeding elements the spacing (S) of the structure (fig. 2). An optical grating alters simultaneously the phase as well as the amplitude of the transmitted, or reflected, light. If the grating alters the phase preponderantly, the structure approaches a phase-grating; if the change of the amplitude predominates the structure approaches an amplitude-grating (8). Phase-gratings are usually transparent objects, the structural elements of which as well as the interstices, are equally transparent to transmitted light. They yield clearly perceptible interference patterns if the refractive index of the elements differs sufficiently from the index of the interstices. In consequence of their uniform transparency these gratings are represented very indistinctly by means of the image-forming arrangement. Amplitude gratings are more or less opaque objects, the structural elements of which are relatively opaque in comparison to the interstice. They may yield clear and distinct images by means of the image-forming arrangement, in so far as the characteristic diameter of the element is still resolved by the objective-lenses.

Regular gratings which have become irregular by distortion, or structures containing a great number of submicroscopic details, generally cause irrecognisable interference patterns with the interferometric constellation, and confused images with the image-forming constellation. These objects can be distinguished by their lustre and the dispersion of white transmitted light. They deflect very irregularly incident rays, if the divergence of the beam is small and often scatter the rays if the divergence of the beam is large. The deviated and scattered rays passing the entrance pupil of an immersion objective yield no recognisable image or may spoil a weak image by superradiation. A recognisable image of these objects can sometimes be obtained by making use of dry objective-lenses, as in that case the glass-air surface of the cover-slip reflects all the scattered or deflected rays with an incidence exceeding 40°. Consequently, all these rays are prevented from entering the object space of the microscope. The relatively small num. aperture of these lenses forms, however, a marked disadventage.

A beam with a small divergence renders an object situated in the object plane of the microscope insufficiently or even not at all selfluminous, consequently, a perceptible image is not formed. If the object, however, proves to be a grating, the rays of the beam are diffracted. The diffracted rays, interfering in the object space of the microscope, produce an image. This image, resulting from the primary interference phenomenon is formed by successive diffraction spectra of rapidly decreasing luminosity. The plane in which this image appears is optically conjugated with the plane of the remoted light sourse, for the figure represents the image of the source altered by the passage of the light through the grating. Rays emerging from this focal image which can be traced back to the same ray, emitted originally by the remoted point source, give rise to a secondary interference phenomenon. The figure, resulting from the interference of these corresponding rays, is formed by a tridimensional afocal light pattern (9). It is this secondary pattern which is observed through the eye-piece. The degree of similarity between the structure of the grating and the interference figure called forth by the structure depends upon the number of the co-operating spectra. The restricted number of these spectra and the intricate relation between the structure and the secondary interference pattern renders it impossible as a rule to recognise intuitively the structure of the grating, or to solve the relationship mathematically. This

difficulty is encountered particularly when the grating is a tridimensional one. Moreover, a tridimensional grating presents a difficulty in so far as the position is not known of the three diffracting planes with respect to the direction of the incident beam. This renders it necessary to turn the mirror of the microscope haphazard until the interference figure flashes out all at once.

In some cases it proved to be possible to recognise the structure of the grating by means of optical analysis of the primary focal image. For this purpose a little instrument, devised by Ambronn and Siedentopf, may be made use of (10). In employing this instrument it is often adventageous to reduce the number of the co-operating spectra. For that reason the fixed exit diaphragm of the central darkfield lens is replaced by an adjustable one. Sometimes the result of the analysis can be verified by means of a microscopic model experiment.

The interferential resolving power of an objective lens is given by the formula,

$$\delta_{min} = \frac{\lambda_0}{A_{obj} + A_{ill}}$$

In this equation δ_{min} means the length of the smallest period of a grating which is still operative in the formation of the primary interference image; A_{obj} the num. operture of the lens and A_{ill} the angular aperture of the incident beam. This last quantity does not exceed 0.1, since the divergence of the beam is very small. It follows from the minuteness of this figure that the minimum distance resolved by a lens is approximately the same for both arrangements. The definition of the diameters resolved by the objective differs, however, in the two cases. It is obvious from this definition that the interferential arrangement is the more far-reaching one, for the length of a period always exceeds the diameter of the element from which the period is built up. It is therefore possible to make a grating distinguishable which cannot be resolved with the image-forming arrangement.

The dissimilarity between the structure of the grating and the secondary interference pattern renders it impossible to derive the periods of the grating direct from the periods of the pattern. When, however, the grating is uniperiodic and the length of the period falls within the limits of the visible light, then the determination of the period becomes possible by means of a monochromator. Beginning from the long red, and turning the monochromator till the interferential pattern flashes up, the magnitude of λ_0 figuring in the formula of the interferential resolving power is determined. The length of the period (δ) can then be calculated by means of this formula.

The interferential method may be combined with the image-forming method, because the central darkfield lens begins to act as a normal lightfield lens of mediocre quality as soon as the divergence angle of the incident beam exceeds a certain limit. In this way an interferential pattern may be superposed upon the image. Starting with the interferential arrangement, and finishing with the image-forming arrangement by gradually increasing the divergence of the illuminating beam is, therefore, sometimes a useful procedure for recognizing the true nature of a microscopic image.

EXPERIMENTAL PART.

The experiments were made with the sartorius muscle of frogs caught during early autumn and kept as far as possible under natural conditions.

Hibernating animals, or frogs having passed a winter in a laboratory, have become so weakened that they cannot be used for these experiments.

The fibres of an entirely fresh muscle are excessively plastic and for that reason easily distorted even by slight mechanical stresses. Consequently they are always deformed when teased out with needles. If the deformation does not exceed certain limits, the original form of the fibre is restored after a short lapse of time. The equilibrium configuration of a fresh, isolated muscle fibre is very nearly a circular cylinder. When a cover-slip is laid upon the fibres they become elliptically deformed. Nevertheless the image of the cross-striation remains unchanged.

Muscle fibres are permeable to light, especially to red and to blue light. The maximum of permeability to red is situated between 640 and 650 millimicra. It constitutes a sharp maximum. The permeability in the region of the blue extends from about 460 to beyond 365 millimicra. It forms a lower maximum, slowly increasing towards the short wave-lengths. Radiations to which the muscle is permeable are always present in the light emitted by the wire of the lamp, particularly the red, if the lamp is burning at low tension. The transmitted light of which a part is absorbed diminishes, within a minute or two, the sharpness of the image formed by the cross-striation. If the alteration of the fibres be observed by means of white light and the interferometric arrangement, the first thing perceived is that the interference pattern, called forth by the structure of the fibre, becomes coarse. Next, the pattern seems to disappear completely and only diffraction disks are seen surrounded by coloured interference rings (11, Fig. 2 B, 3 B) *). The veiling of the pattern through the disks at once disappears when a filter is placed before the lamp only letting through the red radiation to which the fibres are particularly permeable. This proves that the internal structure, causing the interference pattern has remained intact. The whole phenomenon conveys the impression that some component of the fibre coagulates. Probably submicroscopical flakes, or floccules arising in the humoral part of the fibre diffract and disperse the white light.

A method, not essentially new, renders it possible to convert a plastic muscle fibre into a brittle one without materially disturbing either the microscopical structure of the fibre or its characteristic dimensions. For this purpose a fresh and structurally intact sartorius muscle, stretched to its normal length, is put into an aqueous solution of chromalum of 3 to 5 percent. In this solution the consistency of the fibres changes in the course of about two months. The initially plastic fibres become hard and brittle, in many respects resembling a crystal. Like crystals the hardened fibres exhibit definite cleavage planes determined exclusively by their internal structure. These inherent cleavage planes can be reported to a set of three rectangular co-ordinates. The orientation of the co-ordinates with respect to the shape of the fibre may be chosen in such a way that the Z-axis coincides with the longitudinal direction of the fibre, the Y-axis with the tangential and the X-axis with the radial direction, fig. 3. Two of the co-ordinate planes stretch out in longitudinal direction. These are the tangential Y, Z-plane and the radial X, Z-plane. These planes intersect at right angle, provided that the fibres are not deformed and the structure regular. By these planes the fibre becomes split up into layers and fibrils fig. 4 A, B. The fibrils are usually considerd as pre-existent elements. In reality they result from the splitting up of a decaying fibre (12). The

*) The first figure (11) refers to the references, Fig. 2 B, 3 B to the reproductions of the microphotos in that paper.

third co-ordinate plane, the transverse X, Y-plane, is less pronounced in the hardened fibre. It represents the Bowman cleavage plane (13). In an attempt to cut a muscle, hardened in chromalum, on an icemicrotome, the

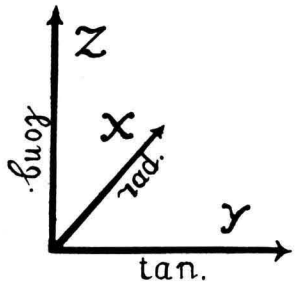

Fig. 3.
Orientation of coordinates with respect to the principal directions of the muscle fibre.
Z: length-direction of the fibre; Y: tangential; X: radial direction.

fibres will be crushed by the knife, and burst. The pieces, resulting from the fragmentation of the fibre, are bounded by the cleavage planes inherent to the structure. Hence the surfaces of a fragment represent the faces of the structure. Most of the fragments are very small, but some are sufficiently large to recognise the cylindrical curvature resulting from the splitting off along a tangential plane. This curvature renders it possible to determine the orientation of the faces with regard to the shape of the fibre. The configuration of the fragments indicates that they have split off from an orthogonal grating. It is for this reason that the cleavage planes can be reported to a set of rectangular co-ordinates. The dimensions of the fragments are determined by the spacing of the cleavage planes. The magnitude of the spacing appeared to be an even multiple of an elementary unit of about 0.5 micron. This unit represents the diameter of a single structural sheet. I never succeeded in splitting up a layer into its two constituent sheets, but it occasionally happens when the fibre bursts.

The internal structure of the muscle fibre can be recognised on the tangential (Y, Z) face of a fragment, but exclusively if the fragment contains but a single sheet. This restriction is necessary in order to avoid axial interferences resulting from the superposition of two or more sheets. Such thin parts occur only near the margin of a fragment. The structure of a sheet can be resolved by means of violet light, an oil immersion system, and the image-forming constellation but the photos are too faint for efficient reproduction. It consists of two sets of point rows, crossing at right angles. This facial image appears in the photos only when the direction of the incident beam is normal to the object plane. The aperture of the beam proves to be of less importance. The structure of the fibre in the X, Z-plane may be recognised in the photos made of the radial face of a fragment. It is also formed by two sets of rectangular intercrossing point rows. The points in both cases are formed by diffraction disks. For that reason the photos allow of drawing no other conclusion than that the points represent the imperfect images of some selfluminous, submicroscopical detail. The diffraction disks, appearing in the photos at the place of the Q-stripe, are clearly distinguishable. The disks appearing at the place of the Z-stripe, are fainter. Hence they make the impression of being smaller than the disks of the Q-stripe. The point rows are equidistant, independently of their nature, whether Z- or Q-rows. There are three successive rows of Q-points, constituting the base of the Q-stripe, regularly alternating with a single row of Z-points, forming the base of the Z-stripe (fig. 5). Three

Q-rows alternating with one Z-row seems to be characteristic of all kinds of animals. This follows clearly from the reproductions of Reitzius' preparations (14).

Measurements made on the photos show that the period of the structure in the longitudinal (Z-)direction amounts to 0.55 micron, in the tangential (Y-)direction to about 0.45 micron and the radial (X-)direction also to about 0.45 micron. The arithmetical mean of the values, measured in the Y-direction and in the X-direction exhibit a slight difference. I shall express this difference by writing 0.45^5 for the dimension of the tangential period and 0.44^5 for the dimension of the radial period of the point rows (fig. 6). It follows from these measurements that the basic cell of the grating is formed by a rhombic prism closely approaching a tetragonal prism (fig. 7). The ratios between the dimensions of the cell are given

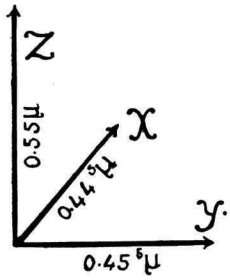

Fig. 6.
Dimensions of the periods in the three principal directions of the grating.

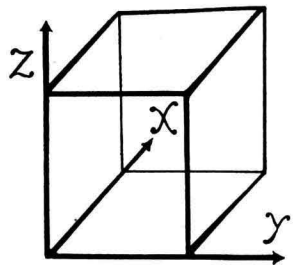

Fig. 7.
Orientation of the basic cell of the grating with respect to the three principal directions of the muscle fibre.

by the continued proportion X: Y: Z:: 1: 1: 1,2. It is evident from the dimensions of the basic cell that the structure of the fibre becomes insufficiently resolved by means of white light. When the spacing of the grating becomes larger, as in the muscle fibres of insects, the structure can be resolved by means of white light and a strong immersion objective, even in histological preparations shrunk by dehydrating agentia.

The diagonal aspect of the grating appears in the photos when a rather narrow incident beam passes in a slightly oblique direction through a fragment with a diameter of about 1 micron. Such a fragment contains only two structural sheets. The pattern formed by the transmitted light consists of two sets of point rows intercrossing at an angle varying between 60° and 80°. Each set of rows crosses the length of the fibre in a diagonal direction (fig. 8). The points arise from axial interference of the rays, deflected by the structure of the superposed sheets (14a). The distance between two successive point rows amounts to approximately 0.65 micron. This magnitude of the spacing, which exceeds the resolving power of the lenses sufficiently, renders it possible to obtain reproducible microphotos notwithstanding that thin fragments are almost as transparent as diatomaceous scales.

In previous experiments this diagonal pattern was at first observed when analysing the primary, focal, interference image caused by a fresh and intact muscle fibre. Because white light was employed the pointrows were not sufficiently resolved by the lens, and the pattern was visible as a linear cross-grating (15, fig. 8). The analysis made it clear that this pattern was not caused by the cross-stripes acting as a non-resolved structural differen-

tiation. It was, therefore, the first indication of the existence of a microscopic grating forming the base of the structure of a fresh and intact fibre. Now it is possible to formulate the relation between the striation of the fibre and the grating. The image-forming constellation in combination with white light does not resolve the Q-rows, and for that reason the three successive rows appear as the dark stripe in the image. The interferential arrangement shows a pattern characterized by a minute striation of the Q-stripe (11, fig. 1). This interferential pattern is caused solely by the rows of the Q-points when the incident beam strikes the muscle fibre in a direction normal to the object plane, i.e. parallel to the X, Y-plane of the fibre. When, however, an incident beam strikes one or more layers of the fibre in a slightly oblique direction the interference pattern assumes the form of a cross-grating.

A comparison between corresponding dimensions of the basic cell of the microscopical grating and of the molecular lattice *) gives the following picture:

$$\text{Z-direction } \frac{2 \times 0.55}{10} \frac{\mu}{\mathring{A}} = 1.1 \times 10^3$$

$$\text{Y-direction } \frac{0.45}{5} \frac{\mu}{\mathring{A}} = 0.9 \times 10^3$$

$$\text{X-direction } \frac{0.45}{4} \frac{\mu}{\mathring{A}} = 1.1 \times 10^3$$

The dimension in the longitudinal (Z-)direction amounts to $2 \times 0.55\ \mu$ because the transverse (X, Y-)layers of the fibre are built up by two sheets each having a diameter of $0.55\ \mu$. The quotients, oscillating round 1×10^3, prove that the basic cell of the molecular lattice is similar in form and similarly orientated as is the basic cell of the microscopic grating. It seems justifiable, therefore, at present to conclude that the difference between the molecular and the microscopical structure can be reduced to a simple difference in the scale of realisation of both structures, the ratio between the two scales being of the order of magnetude of 10^3. From this point of view it becomes evident that a cross-striated muscle fibre is uniaxial double refractive since its structure closely approaches a tetragonal prismatic crystal, and that the amount of the double refraction depends on the directional differences in the density and tension actually existing in the structure.

A comparison between the molecular structure of the muscle fibre and the molecular structure of an artificial multilayer film of corresponding dimensions, built up by Astbury from its molecular constituents, leads to a result different from current opinion (16). It makes probable that the 10 Å dimension of the muscular lattice (Z-direction) is the sidechain period, the 5 Å dimension (Y-direction) the mainchain period and the 4 Å dimension (X-direction), usually indicated as the backbone spacing of the muscular lattice, the intermainchain period. The stability and unfolded condition of the sidechains explains the resistance of a muscle fibre against longitudinal tension and in the same way the difficulty to divide a fibre into Bowman disks strikingly contrasting with the readiness with which the fibre splits up in longitudinal fibrils.

*) In order to avoid confusion the term "lattice" is used when dealing with the molecular structure of the fibre and "grating" in the case of the microscopical structure.

When a fresh, intact muscle fibre is placed under the microscope the fibre begins to decay soon after the passage of the light. This causes no surprise, since the intensity of the light necessary for observation is very considerable. One of the first symptoms of the decay is the length striation of the fibre caused by the splitting of the grating into fibrils (11, fig. 3 A). Measurements taken on photos prove that the spacing of the striation amounts to about 2 micra. Muscle fibres derived from weakened animals and somewhat carelessly isolated, split up almost immediately after the light has passed. Consequently the intact state of the grating escapes observation. This has led to the generally accepted opinion that a muscle-fibre is composed of "primitive fibrils" held loosely together by a sarcolemma. Carefully isolated fibres of strong animals remain intact, however, for several minutes and then show no trace of a longitudinal striation (12). The transverse striation resulting from the splitting up of the fibre in that direction is not so distinctly recognizable as the longitudinal striation. The first transverse-stria to appear runs close alongside the Z-stripe (17). It accentuates the image of the Z-"membrane" called forth by the row of Z-points. The spacing of this transverse striation also amounts to about 2 micra fig. 5 B. At a more advanced stage of decay the fibrils formed first split up further and the spacing of the longitudinal striation is reduced to about 1 micron, fig. 4 B. The transverse stria halving the initial spacing runs through the middle of the Q-stripe, close along the middle row of the Q-points. This stria is usually described as a mesophragm. A prolonged observation of the formation of this stria proves without any doubt that it is the image of a narrow cleft, filled with fluid in which small spherules may be seen, when a paraboloid darkfeld condensor is applied. The swelling of the fibre accompanying its progressive decay prevents seeing if the structure splits up further. As far as observation goes it is obvious that a fresh decaying fibre splits up into units, the dimensions of which are even multiples of about 0.5 micron. The location of the clefts seems to depend on the spacing of the structure in like manner as in a hardened fibre. Hardening a fresh muscle fibre is evidently a method for rendering the splitting up of the grating more easily observable and at the same time probably more regular. Not only the hardening of a tissue by means of a fixating fluid, but each action interfering with the natural conditions of a tissue, invalidates the stability of the grating. This always leads to splitting up the grating along its inherent cleavage planes. Microscopic observation is, therefore, inevitably accompanied by a more or less pronounced cleavage of the structural part of the tissue.

In a final set of experiments I have tried to obtain some information about the nature of the selfluminous points discernible on the photographic plate as diffraction disks. The experiment is based upon the observation that a fresh and intact muscle fibre remains in osmotic equilibrium with a Ringer solution having a freezing point depression of 0.81° C and a P_H of about 7.2, but that a fresh and cross-sectioned fibre rapidly swells up and distintegrates in this solution. In consequence of the swelling the interior of the fibre bulges out through the aperture of the cross-section. If, on the contrary, a cross-sectioned fibre is put into a buffered solution having the samen P_H as the interior of the fibre, a slight retraction takes place near the surface of the cross-section (18). Consequent on this retraction the cross-section becomes smaller and slightly excavated, and the striation near the margin of the section narrower (fig. 9). The citrate buffer of MacIllvaine with a P_H of 6.8 proved to be a suitable buffer (19). The depression of the freezing point of the buffer amounted to 0.43° C.

Hence it is a non-iso-osmotic solution in which the structural part of the fibre remains some time unchanged. This temporary stability of the structure makes it possible to cut a fresh fibre into thin disks with a Gillette blade and to observe the surface of the disk (X, Y-plane) in contact with the buffer. The disks, if they are sufficiently thin, strongly scatter transmitted light in consequence of the distortion of the grating caused by the cutting of the fibre. For this reason it is only possible to obtain a recognizable image of the surface by means of a dry objective lens. With such a lens it will be clearly seen that the surface of the cross-section is not smooth but has been ravelled out by the teeth of the knife. The ravels are triangular with their base still in connection with the surface. The point of the ravel, extending into the buffer solution, performs lively brownian movements. In the course of a few hours the ravelling of the surface disappears in consequence of the disintegration of the ravels, and the surface becomes smooth and regular. When now an incident beam, with a small divergence angle passes through a disk in a slightly oblique direction, the diagonal aspect offered by the transverse (X, Y-)plane of the grating, becomes recognizable. The interference pattern, where this is distinctly discernible, is formed by intercrossing short shiny tracts (fig. 10). By this arrangement the pointrows appear as tracts, as the distance between the points is too small to be resolved by a dry apochromatic lens. It is now possible also to follow the course of the disintegration of the ravels by means of a paraboloid dark-field condensor and a strong immersion objective. With this arrangement it can be seen that the point of the ravel breaks up into short thread-like elements. As soon as these elements become free in the buffer solution they execute brownian movements. When moving they faintly scintillate. After a short lapse of time a part of these scintillating, thread-like fibrils adhere to the coverslip, whilst others cling together and form small irregular coagula. These coagula, as far as they can be seen with the image forming arrangement, appear as more or less ellipsoid-shaped granules. The elements, adhering to the coverslip, perform pendulating brownian movements, which may persist for several hours. At the place where they adhere to the coverslip a diffraction disk is visible, surrounded by segments of interference-rings. It follows from the similarity of the diffraction figures, and also from the way in which the light is scattered, that the ultimate element resulting from the disintegration of the grating is formed by thread-like fibrillae of approximately equal lengths and scattering power. The light scatterd by the adhering fibrils resembles a sweeping bundle emitted by a swinging phare. The J-spherules, which are always easily recognizable when the disintegration of a cross-section takes place rapidly in a phosphate solution, cannot by any means be rendered visible in this citrate buffer. It follows, in my opinion, from the manner in which the structure disintegrates that the grating is built up of submicroscopic, thread-like fibrillae and that the diffraction disks represent the imperfect images of the knotpoints of this fibrillous grating.

Regular gratings, of which the periods are commensurable with the wave-length of the visible light, are very liable to cause interferences even under optical conditions in which interference is not suspected. These interferences, superposed upon the microscopic image of the object, falsify the image. The traditional picture of the cross-striated muscle fibre forms an example of such a complex image. The cross-striation of the fibre acting as a microscopically non-resolved structural differentiation may give rise to an interference phenomenon when a slight inclination is

imparted to the incident beam with respect to the plane of the striae (X, Y-plane). The spacing of the pattern corresponds to the spacing of the cross-striation. The superposition of this pattern upon the microscopic image accentuates the image of the cross-striation of the fibre. If the focus of the microscope is but slightly varied, the position of the interferential light maxima and minima becomes interchanged. In this position the maxima correspond to the dark (Q-)striae and the minima to the light (Z-)striae. This reverses the relative brightness of the stripes. At an intermediate position of the focus the cross-stration apparently vanishes. The spacing of the interferential pattern at once jumps to one half of its initial value when the inclination of the incident beam is but slightly increased. The superposition of this pattern upon the image of the cross-striation produces pictures described as the "narrow striation" of the fibre (fig. 11). The interferential method further brought to light that the minute, longitudinal striation of the Q-stripe is a result of interferences caused exclusively by the Q-points of a fresh and intact muscle fibre (11, fig. 1). In this case it did not prove feasible to superpose a sharp interference pattern upon a sharp image (fig. 12 A) notwithstanding this the photo shows clearly the position and the origin of the interferences.

In a previous paper published several years ago I gave a description of the structure of the striped muscle fibre based exclusively upon images afforded by histological preparations (20). The application of the interferential arrangement made it clear that this description was based upon the observation of the cleavage planes inherent to the structure of the fibre. For this reason the picture, resulting from these earlier observations, agrees with the picture obtained by means of more refined optical methods. The advance made in these methods lies in the recognition of the grating as the structural base of the fibre. This renders the interpretation of the observations more simple, and at the same time it established an analogy between the structure of the fibre and the structure of a crystal.

In histological preparations the clefts, resulting from the splitting of the structure, are filled up with Canada balsam or some other highly refractive substance. These thin layers of balsam refract and reflect strongly the transmitted light, and in this way convey the impression of highly refracting membranes. The walls of the clefts, formed by the faces of the structure, are not perfectly smooth. Hence they scatter light irregularly and create the illusion that these apparent membranes are of measurable thickness. The walls of the clefts, and particularly the places where two cleavage planes intersect, very readily absorb dyes (e.g. reduced silver, hematoxyline). This strengthens the impression that the clefts represent pre-existing structural differentiations.

The dimensions of the basic cell forming the geometrical unit of the grating and the dimensions of the unit, delimited by the microscopic cleavage planes may be different. If they differ, the dimensions of the units resulting from the splitting of the structure are multiples of the corresponding dimensions of the basic cell. This is due to the fact that the fibre never completely splits up when the tissue is hardened. The incompleteness of the spliting may be seen when the images offered by a section of a hardened fibre stained in a suitable manner with hematoxyline be drawn exactly plane-for-plane (21). In each plane of the preparation the narrow blue lines indicating the clefts, are short, and everywhere interrupted. If however, a sufficient number of drawings made at successive planes are superposed, a coherent image of the structure can be obtained. A microscopist who keeps turning the screw of his microscope is actually doing the same, but

he mentally integrates what he is observing in the successive planes. Therefore pictures made in this way by skilful observers who critically eliminate what appears alien to the structure, yield reliable projection images of the structure based upon the observation of the cleavage planes.

The analysis of the structure of the sarcoplasmatic endplate by means of plane-for-plane drawings proved that the terminal net forms the plane projection of a regular tridimensional structure. Rhombic pyramids appeared to be the units into which the sarcoplasm splits up. This suggests that the basic cell of the sarcoplasmatic grating is formed by a bodily-centred prism, for these prisms may split up into pyramidal elements owing to the connections between the centre and the corners of the prism (fig. 13). By following this suggestion the picture to be made of the structure of the muscle fibre becomes extremely simple. A continuous rhombic grating, common to the contractile and to the sarcoplasmatic part

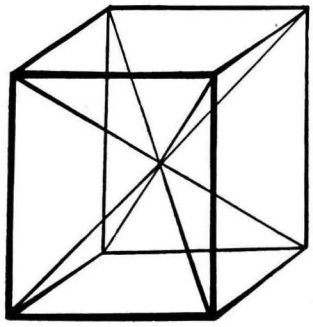

Fig. 13.
Rhombic bodily centred prism.

of the fibre, constitutes the structural base of the whole muscle fibre. A plane surface, dividing the simple prismatic elements from the bodily centred elements, forms the limit between the contractile part and the sarcoplasm. In sections parallel to the length of the fibre, and passing through the endplate this plane appears as a straight line (22). The differentiation of the contractile substance results, therefore, from a regressive simplification of the sarcoplasmatic grating. In agreement with this structural simplification is the reduction of the number of possible dynamical states of the muscle fibre. The all-or-non rule points out that there exists only two of these states, the stretched and the shortened state of the fibre.

A model made of the transition between the contractile substance and the sarcoplasmatic endplate shows how the prevailing cleavage of the sarcoplasm along diagonal faces may convey the impression as if a set of parallel triangular furrows, extending in the longitudinal direction of the contractile part of the fibre, receives the triangular teeth of the similarly shaped adjacent surface of the sarcoplasm (fig. 14). The spacing of the diagonal period of the sarcoplasmatic grating amounts to about 0.35 micron. This dimension, being below the resolving power of the lenses, proves that the meshes of the sarcoplasm which are microscopically observable as the meshes of the terminal net, are multiples of the units characterizing the grating. Pictures made by plane-for-plane drawings often make the impression that cleavages may also occur along hemihedral faces. This fact also seems to have been observed by others (23).

The transition of the axoplasm of the motor nerve into the sarcoplas-

matic endplate takes place by a continuous deformation of the stretched geometrical elements of the axoplasmatic grating into the approximately equilateral elements of the sarcoplasmatic grating. A flat, unicellular layer of large cells, obviously belonging to the endplate, is situated at the transition of the sarcoplasm into the contractile substance. In a longitudinal section these elements may be seen as a single row of elliptic cells adjoining the contractile part of the fibre. These cells are possibly connected with the conveyance of the nervous impulses to the contractile substance. The sympathetic fibre on the other hand retains its individuality on its course through the endplate. Here the fibre terminates in or round a small cell (24, fig. 1). This cell seems to have close contact with the contractile part of the fibre. By the intermediation of this cell the sympathetic fibre may perhaps influence the humoral constituent of the muscle fibre (25). The discrimination between the motornerve and the sympathetic nerve has become possible by means of the interferential method. The period of the pattern called forth by the axoplasmatic grating of the motor-nerve amounts to about 0.8 micron, and the period of the axoplasm of the sympathetic nerve to about 0.4 micron. The ratio of the two periods is, therefore, approximately as 2 : 1, (24, fig. 1, 2).

Rhombic face-centred prisms, splitting up into dodecahedrons would also seem to occur in living nature according to the researches of Seifriz (26).

SUMMARY.

1. Fresh muscle fibres of the frog (M. sart. of R. esc.) are extremely plastic and therefore easily deformed when teased with needles and isolated. Isolated fresh fibres, when intact, remain temporarily unaltered in Ringer solution (P_H 7.2); when cross sectioned, the cut surface swells up and quickly disintegrates. In a buffered solution of MacIllvaine (P_H 6.8) the cut surface slightly retracts and remains temporarily unchanged.

2. A fresh muscle is very sensitive to transmitted light, particularly to red light in the neighbourhood of 640 millimicra wave-length and to blue light, beginning in the neighbourhood of 460 millimicra and extending in the ultraviolet beyond 365 millimicra.

3. The two components of the muscle fibre, the structural part and the humoral part, react differently to transmitted light. The structural part formed by a rhombic, fibrillous grating, proved to be the more stable component of the fibre and remains temporarily intact. The humoral part, formed by the fluid by which the grating is soaked, coagulates shortly after the passage of the light.

4. Common to the contractile part and to the sarcoplasmatic part of the muscle fibre is a rhombic grating. This orthogonal grating, as far as it forms the base of the contractile part, is built up of simple prismatic elements; the grating forming the base of the sarcoplasmatic part is built up of bodily centred prismatic elements. The transition of the two types of elements takes place along a plane surface.

5. The axoplasmatic grating of the motor-nerve passes over continuously into the sarcoplasmatic grating. The grating, forming the structural part of the sympathetic nerve retains its individuality on its course through the endplate and terminates in, or round, a small cell situated at the transition of the sarcoplasm into the contractile substance.

6. A sartorius muscle can be hardened in a solution of chromalum. The

hardened fibres resemble in many respects a brittle crystal. The dimensions of the grating, forming the common structural base of the fibre, are thereby not markedly altered. The sidelength of the basic prism of the grating amounts to 0.55 micron in the longitudinal direction of the fibre, to 0.45^5 in the tangential direction and to 0.44^5 in the radial direction. The basic prism, therefore, closely approaches a tetragonal prism.

7. When a hardened muscle is cut on an ice-microtome the fibres are crushed by the knife. The fragments resulting from the bursting of the fibre split off along cleavage planes inherent to the structure of the grating.

8. The basic cell of the microscopic grating and the basic cell of the molecular lattice would seem to be similar and similarly orientated with respect to the shape of the fibre. The ratio between corresponding diameters is of the order of magnitude of 1×10^3. Fibres exhibiting this structure closely resemble double refracting, uniaxial crystals. The amount of the double refraction depends in that case upon the directional differences in the density and in the tension actually existing in the structure.

9. The regularity of the grating and the dimensions of its spacings which are commensurable with the wave-length of the visible light, render this structure very liable to cause lateral as well as axial interferences.

10. The traditional picture of a striped muscle fibre is a complex image resulting from the superposition of an interference pattern, called forth by the cross-striation upon the image of the fibre.

11. The diagonal aspect of the grating simulating a cross-grating results of interferences produced by the orthogonal grating under particular optical conditions.

12. Every action interfering with the natural conditions of a tissue invalidates the stability of the structural part of the tissue. If this structural part is formed by a grating of microscopic dimensions, histological methods tend to make visible the clefts resulting from the splitting up of the grating along its inherent cleavage planes. Plane projection-images of the clefts afford reliable information respecting the structure of the grating based upon the observation of the cleavage planes.

May 1942.

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The difficult cricumstances, still existing in this country, prevented the microphotos to be reproduced by means of a more appropriate technique.

Fig. 4.

A: Exp. of 10.12.40. Fragment of a muscle fibre hardened in chromalum split off along a transverse (Y, X) cleavage plane. The fibre is split up into layers and fibrils of abouth 4×0.5 micron. Wide opened incident beam, violet filter. Magn. $500 \times$; Enl. $2 \times$; $1 \mu = 1$ mm.

B. Decaying fresh muscle fibre. The fibre splits up in fibrils of about 2 and of 1 micron diameter. The arrow indicates the length (Z-) direction of the fibre. White light; condensor diaphragm wide opened. Magn. $500 \times$; Enl. $2.5 \times$; $1 \mu = 1.25$ mm.

Fig. 5.

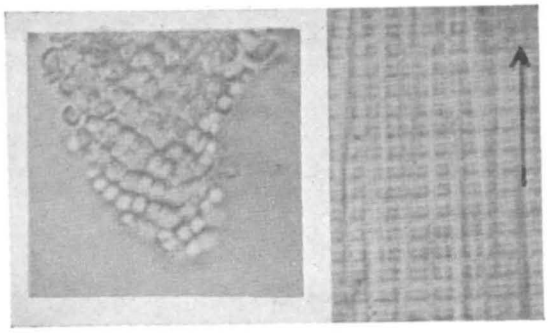
A. Opaque tridimensional model of the contracttle part of the grating. Stretched state of the fibre. The white, circular spots represent the diffraction disks called forth by the Q-points; the grayish spots, the diffraction disks of the Z-points. Scale $1 \mu = 7.5$ mm.

B. Negative of model A. In this model black lines indicate the inherent cleavage planes of the grating. Arrow indicates length (Z-) direction.

Fig. 8.

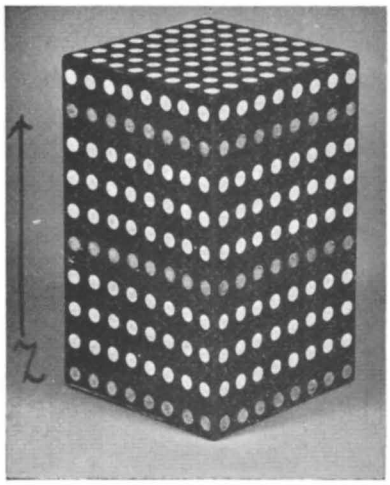
A. Exp. of 11.11.41. Tangential (Z, Y) fragments of a fibre hardened in chromalum. Fragments composed of two sheets. Diagonal aspect of the grating. Magn. $400 \times$; Enl. $2.5 \times$; $1 \mu = 1$ mm. Interferential arrangement, violet filter.

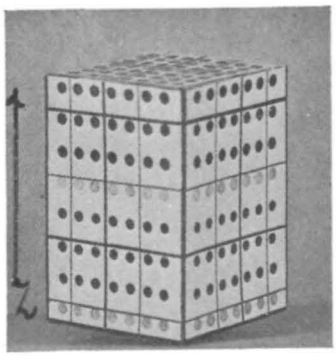
B. Exp. of 28.11.40. Tangential (Z, Y) fragment of a fibre hardened in chromalum. Diagonal aspect of the grating near the margin of a thick fragment. Interferential arrangement, violet filter. Magn. $400 \times$; Enl. $2.5 \times$; $1 \mu = 1$ mm.



A B

Fig. 4.





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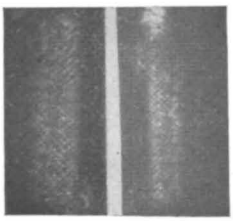
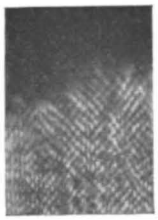
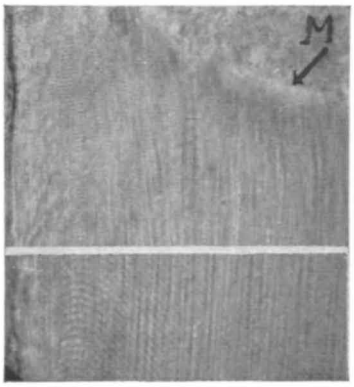




A B

Fig. 8.



A

B

Fig. 9.

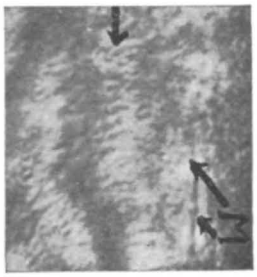
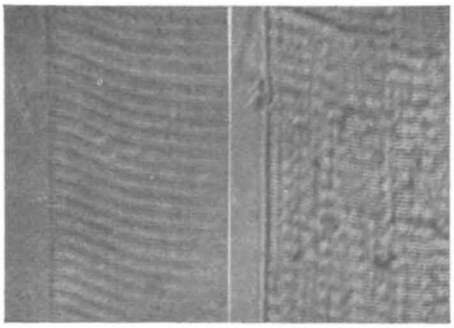


Fig. 10.



A B

Fig. 11.

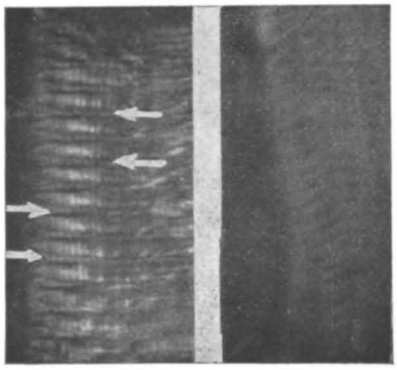


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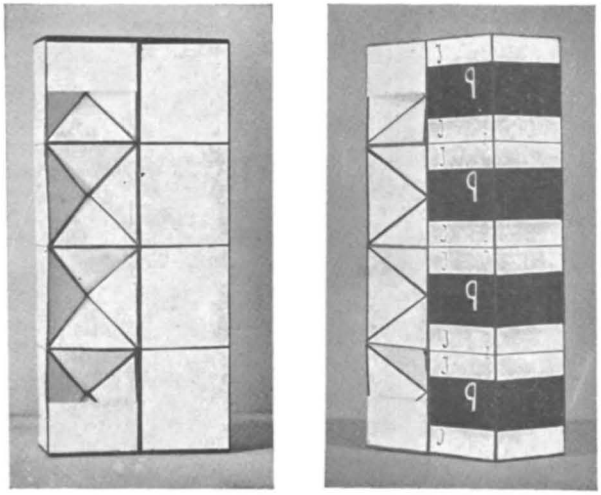
Fig. 12.



A B

Fig. 14.

Fig. 9.

Exp. of 17.10.40. Gross-sectioned fresh muscle fibre in a MacIllvaine buffer (Ph 6.8).

A. Narrow striation caused by local contracture of the fibre. Arrow-M points to the margin of the cross-section.

B. The same fibre, 0.5 mm further. Normal striation at the left of the image. Green filter; dry apochromate. Magn. 250 ×; Enl. 2 ×; 1 μ = 0.5 mm.

Fig. 10.

Exp. of 31.10.40. Cross-section of a fresh muscle fibre in a MacIllvoine buffer (Ph 6.8). The three arrows point to diagonally intercrossing tracts. M indicates the margin of the section. The tracts represent the axial interferences called forth by the superposed, transverse (X, Y-) layers of Q-points. The separate points of the interference pattern are not resolved by the lens and therefore appear as continuous tracts. Narrow incident beam; green filter; dry apochromatic lens. Magn. 250 ×; Enl. 3 ×; 1 μ 0.75 mm.

Fig. 11.

Exp. of 10.2.41. Tangential (Z. Y) fragment of a muscle fibre hardened in chromalum.

A. Microscopic complex image of the fragment, normal cross-striation. The period of the superposed interference pattern corresponds with the period of the cross-striation. The image predominates.

B. Complex image of the fragment at the same spot. Superposition of an interference pattern halving the period of the cross-striation ("narrow striation"). The interferences are made the predominant feature in he picture. Green filter. Magn. 500 ×; Enl. 2 ×; 1 μ = 1 mm.

Fig. 12.

A. Exp. of 6.2.41. Tangential (Z,Y) fragment of a muscle fibre hardened in chromalum. Interference pattern caused by Z, Y-layers of Q-rows, superposed upon the image of the fibre. The Z-"membrane" indicated by white arrows predominates in the complex image. The picture shows that the interferences are located at the place of the Q-rows. Interferential method with a rather widely opened incident-beam. Green filter. Magn. 400 ×; Enl. 4 ×; 1 μ = 1.6 mm.

B. Fresh and intact muscle fibre. The dark (anisotropic) Q-stripes finely striated. The period of the striation amounts to about 0.6 μ. This interference pattern is caused by the rows of Q-points. Interferential arrangement; magn. 400 ×; Enl. 2.5 ×; 1 μ = 1 mm.

Fig. 14.

A. Opaque tridimensional model of the transition zone between the sarcoplasmatic endplate and the contractile part of the fibre. Aspect of the radial (Z X) fase. Part of the wall, at the side of the sarcoplasm, is cut a way in order to show the connection between the bodily centred, prismatic elements of the sarcoplasm and the simple prismatic elements of the contractile substance.

B. The same model as 14 A, but now turned about 45°. In this position the model corresponds to a longitudinal section intermediate between the radial and tangential fase of the structure. The model shows the apparent connection between the meshes (faces of the bodily centred, prismatic elements) of the sarcoplasm with the Q-stripes of the contractile substance.