**Medicine.** — *The ultimate structure of the striped muscle fibre of the frog distinguishable with the microscope.* By J. W. LANGELAAN.

(Communicated at the meeting of November 29, 1941.)

On a previous occasion I gave a short account of my researches on the structure of the muscle fibre, and especially drew your attention to an interferential pattern caused by a fresh and structurally intact fibre. This pattern consists of a fine, narrow striation of the *q*-stripe. White dashes alternate regularly with small dark strokes. The period of this pattern approximately amounts to 0.7 micron. The white dashes represent the light maxima, the dark strokes the minima of the interference figure. The *I*-stripe presents no recognisable structure with the interferential arrangement of the microscope. The interference pattern forms the only indication of the existence of a regular structure of the fibre, of which the characteristic dimensions are commensurable with the wave-length of the visible light.

The interferential method, by which the pattern can be rendered visible, forms a strict application of ABBE's theory on microscopical vision. This spectrum theory has been laid down in a compendious, mathematical form by LUMMER and REICHE [1]). Unfortunately these authors dealt only with the problem of twodimensional gratings, but the muscle fibre is a tridimensional one. The method consists of the appliance of a narrow incident beam of which the divergence angle is equal to, or smaller than, 3°, monochromatic light, and a darkfield objective-lens according to the method of SPIERER [2]). The small opaque shield upon the curved surface of the front lens of the objective screens off the central bundle (spectrum of zero order) of the incident beam diffracted at the object. In this way the darkfeld is made. An adjustible diaphragm, forming the exit-pupil of the objective, makes it possible to restrict the number of the spectra which co-operate in the formation of the interferential pattern.

The analysis of the primary interferential image, present in the neighbourhood of the principal focal plane of the objective, indicates that the interferential pattern, called forth by the microscopic structure of the muscle fibre, arises from the joint effect of two intercrossing gratings. The elements of which these patterns are composed run in a diagonal direction in respect of the length of the fibre. They intercross at an angle varying between 60° and 80°. When the interferential pattern is screened off by a diaphragm, placed in the neighbourhood of the principal focal plane of the objective, a vague image remains of the ordinary cross striation of the muscle fibre. The diameter of the alternating light and dark stripes amounts to about 1 micron. This image becomes more distinctly visible when the divergence of the incident beam is increased and the exit diaphragm of the objective widely opened. This image obviously results from a periodic variation in the density of the fibre, causing a variation in the permeability of the tissue to light. The structure of this differentiation is submicroscopic, for it cannot be distinguished with the microscope, nor does it give rise to observable interferences.

A method, not essentially new, renders it possible to convert a plastic muscle fibre into a brittle one without materially disturbing the microscopical structure of the fibre nor its characteristic dimensions. For this purpose a fresh and structurally intact sartorius muscle, extended to its natural length, is put into an aqueous solution of chromalum of 3 to 5 percent. In this solution the plastic fibres become hard and brittle in the course of about two months. If, now, one tries to cut the muscle on an ice-microtome, the fibres are crushed.

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1) LUMMER und REICHE, Lehre v. d. Bildentstehung i. Mikroskop, 1910.

2) SPIERER, Ch. Koll. Zeits. **51** (1), 162—163 (1930); **53** (1) 88—90 (1930).

by the knife and burst. This always happens independently of the direction in which the muscle is cut.

The fragments, resulting from the bursting of the fibres, prove to be split off along preformed cleavage planes, in the same way as a brittle crystal would do under similar circumstances. Most of the fragments split off along a plane parallel to the surface of the fibre. This plane represents the tangential cleavage plane. The smaller side of these fragments is formed by the radial cleavage plane, whilst the third side is formed by the transverse, or BOWMAN, cleavage plane. The directions of these planes are determined by the internal structure of the fibre. They represent the faces of the structure. The configuration of the fragments indicates that they have split off from an orthogonal grating. The measurement of the length of the sides of the fragments proves that they are all even multiples of a unit length of about 0.5 micron. This dimension represents the diameter of a single structural leaf of the grating. It follows from this observation that the layers of which the grating has been built up are each composed of two leaves. Near the margin of the fragments small zones often occur, of which the diameter is about 0.5 micron. These thin, single-leaved edges arise through the bursting of the fibre when crushed by the knife. I never deliberately succeeded by means of needles or other mechanical contrivances in splitting up a layer into its leaves.

The single-leaved edges present no distinguishable structure when observed by means of white light and the image-forming arrangement of the microscope. Photos, however, made by means of violet light ($\lambda = 445$ millimicra) and an incident beam with a rather small divergence angle, show the structure of the leaf. The image present on the photographic plate consists of two sets of point rows, intercrossing at right angles. The points are equidistant, having in both directions a period of about 0.5 micron. The same observation can be made on the radial face of the fragments. The points visible on the photos are diffraction (AIRY) disks. They represent therefore the images of some submicroscopical detail, made self-luminous by the light of the incident beam. The real structure of the detail remains, of course, undetermined.

The photograms can be measured, and prove that the period of the point row in the longitudinal direction of the fibre amounts to 0.55 micron. The period in the tangential and radial directions varies round 0.45 micron. The arithmetical means of the measurements in these directions indicate, however, that the tangential period is slightly greater than this figure, and the radial period slightly less. This may be indicated by writing for the tangential period $0.45^5$ and for the radial period the figure $0.44^5$. The basic cell of the microscopic grating is, therefore, a simple rhombic prism, closely approaching a tetragonal one. It must, however, be emphasized, that this represents the basic cell of the contractile part of the fibre for the basic cell of the sarcoplasmatic part is slightly more complicated.

The description of the structure of the microscopic grating has been based upon images presented by a single structural leaf, for this excludes axial interferences. As far as I know axial interferences have always been neglected notwithstanding that they may produce interferences under conditions, making interference entirely unexpected. For that reason axial interferences superposed upon a microscopic image are not recognised. Lateral interferences are well known and also treated theoretically, though their effects often fail to be recognised [3]).

Experiments made by means of an appropriate buffer-solution, render it possible to follow the desintegration of the microscopic grating for several hours. In this solution the traces of but one element becomes visible, when using a paraboloid darkfield condensor. The only element which becomes observable in this buffer is a short thread-like fibril of which the characteristic diameter is submicroscopic. For that reason it cannot be seen with the image-forming constellation. A great number of these fibrils, still bearing their negative electric charges, adhere to the coverglass, other ones, after having lost their

[3]) RAYLEIGH, Scientific Papers, IV (222), 235—260 (1903).

charges, cling together and form small, irregularly shaped granules. The largest of these granules are of microscopic dimensions. The fibrils, adherent to the coverglass, execute brownian, pendulating movements. The similarity in shape and dimension of the diffraction figures, caused by the moving fibrils, render it probable that they have all approximately the same length and diameter.

The most probable conclusion to be drawn from these experiments is, now, that the muscle fibre forms a tridimensional, rhombic grating, closely approaching a tetragonal grating. The dimensions of the three periods, characterising the grating, approach the limit of the resolving power of the objective-lenses. The elements composing the grating are most probably thread-like filaments. The submicroscopic knot-points of this filamentous grating can be made self-luminous. These self-luminous points appear in the image as the diffraction disks, composing the orthogonal point-rows observable in the photograms made by means of violet light and the image-forming arrangement.