MICROSCOPIC INVESTIGATION OF MUSCLE FIBRIL TURNED ON ITS LONGITUDINAL AXIS.

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With 4 Figures in the text.

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The smallest unit in the cross-striated muscle observable with light microscope is the muscle fibril, in which dark and light striations follow each other, that is to say, seen in polarized light, successive isotrop and anisotrop segments. Numerous investigations have attempted to reveal the structural cause of this optical cross-striation. In classical opinion it was caused by the fibrils' being made of two different materials which, making discs, alternated with one another.

But beside the classical opinion, the view has often been expressed that the fibril's striated appearance was not due to differences in substance but to an optical illusion resulting from the geometric placing of the sub-microscopic particles — the micels — which are part of the fibrils' construction (HÜRTLE, 1931).

In recent years SZENT-GYÖRGYI (1940—44) and his co-workers (I. BANGA, K. BALENOVIC, F. B. STRAUB) broke up the muscle's contractile substance into its 2 components, myosin and actin.

The union of the 2 substances according to stöchiometrically determined proportions creates the contractable actomyosin. On the basis of data on the components' physico-chemistry and observations from the standpoint of place-filling, mechanism and function, SZENT-GYÖRGYI (1946) attributes the spiral structure to acto-myosin.

As the spiral structure causes a certain periodicity in the substance's geometrical placing, it was natural to think that perhaps the cross-striation too could be brought in relation with the spiral structure of the actomyosin threads running through the fibrils. (SZENT-GYÖRGYI, l. c.)

The problem can be decided on the basis of the following considerations: If the fibril is made of discs then when it turns the cross-

striation would always have to stay in the same place. But if the spirals extend throughout the fibrils, then in turning, while the optical picture remained unchanged, the cross-striation would have to shift.

The turning of the fibrils was attempted by SZENT-GYÖRGYI, later by GERENDÁS, and it was established that when the fibril is turned the cross-striation does in fact move in a way corresponding to the above assumption. But, due to technical difficulties, it was not possible in a single case to follow the shifting of the cross-striation throughout the complete rotation of the fibril. Furthermore, the optical changes caused by the alteration in distance from the object (see below) were not taken into account.

In the present investigations we intended primarily to eliminate the changes in the microscopic image arising from microscopic depth of the fibril, furthermore, by developing the rotating technic, to follow the behaviour of the cross-striation throughout the fibril's complete rotation and to demonstrate it by microphotographs.

EXPERIMENTAL MATERIAL AND PROCEDURE.

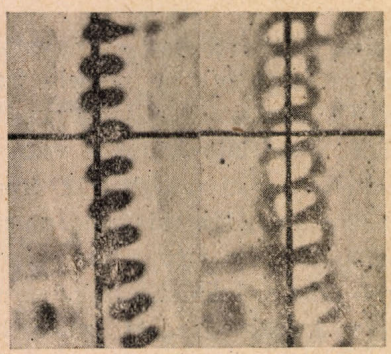
For the experiments the flying muscles of the thorax of a water beetle (Hydrophilus) were used. The preparation of the muscle was carried out after a method similar to SZENT-GYÖRGYI's. The flying muscle was fixed in 1% osmic acid, after which it was finely minced with a sharp knife. The minced muscle was shaken for 30 minutes in frog-Ringer solution with glass pearls, then centrifuged. On this mechanical interference the fibres separated into fibrils. The centrifuge was mixed with a 10% slightly warmed gelatine and then, while still lukewarm, sucked into a capillary glass tube. After the gelatine had stiffened the capillaries were microscopically examined and those selected in which single fibrils were centrally placed and their long axis parallel with the long axis of the capillary. For rotating the capillary a Zeiss capillary rotator was used, attached to the object table of the microscope. The area between the capillary and the lens was filled with cedar oil. This instrument assured that during the rotation the capillary made no excentric movement, it prevented the deplacement of the capillary along the axis, and its division in degrees made possible the constant control of the angle of rotation. The photographs were made with a Contax machine mounted on a (Reichert) microscope, with the addition of an observation apparatus consisting of a mirrorprism and side observation tubes. The photographs were made with an enlargment A=0,75, 60x

and 10 x photo-ocular. The most difficult task was to eliminate the movement of the different parts of this installation. We achieved great stability by fixing the microscope, the photographic appendages and the Contax apparatus on a steel rod resting on a wide base. With this the rotator could be turned, the film rolled and exposures made without the slightest movement of the contraption.

EXPERIMENTAL RESULTS.

a.) The change in the optical image due to alteration in distance from the object.

SCHÄFFER (1922) in his description of cross-striation of muscle fibre announces the phenomenon that if we move the lens of the microscope from a higher to a lower situation the Q striation changes places with the I striation. In our investigation we found the same appearance in the fibrils (Figure 1).



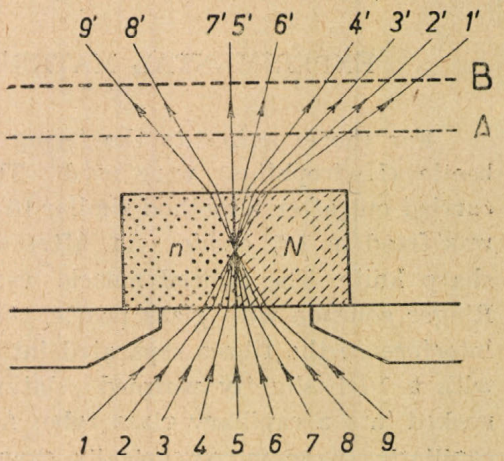


Fig. 1. An isolated wing-muscle fibril. Shifting of the cross-striation caused by moving the lens of the microscope to higher (A) or deeper (B) situation.
Fig. 2. Explanation of *Becke's* phenomenon.

The observed change of striation is to be explained by BECKE'S phenomenon (Figure 2). It is essentially that: The condensor of the microscope projects a conical light-bundle on the object. The path of the light-bundle is influenced partly by the reflection, partly by the refraction at the point of contact of the different refractive media of the object. Let the refractive index of the two media be n, N respectively, and $n < N$.

Investigating the surface of contact of the two different refractivable media through the microscope a part of the light rays arriving from the microscope mirror penetrate from the medium N into medium n, another part from n into N. In consequence of the total reflection the greater part of the light rays get into N medium, and only a smaller part continues to go through n medium. (8, 9) Those rays which reach the N medium at a greater angle than the limit-angle of the total reflection are reflected on the contact surface and remain in the same medium. (6, 7). Of the rays arriving from the N medium only those can penetrate in the n medium, whose angle of incidence is less than the angle of the total reflection. On the other hand, the rays reaching n medium from the microscope mirror can all penetrate into N medium. (1, 2, 3, 4).

For this reason the intensity of the light increases on the border of the N medium (the rays condense there), that is, there appears a narrow light stripe, BECKE's line.

When we move the lens of the microscope to a higher situation the light-stripe widens in the material which shows greater refractive index and it seems as if it moved to the interior of this medium.

In the case of the fibril, the anisotropic striation (Q) is the medium of greater refractive index, the isotropic striation of less. Moving the lens to a higher situation a light-coloured stripe begins to move from each side of the anisotropic striation towards its interior. These light-stripes cross each other at various heights and at these points increase the light intensity. (Figure 3). Thus, due to the crossing of light rays, the light stripes appear above the Q striation in situation A and above the I striation in situation B.

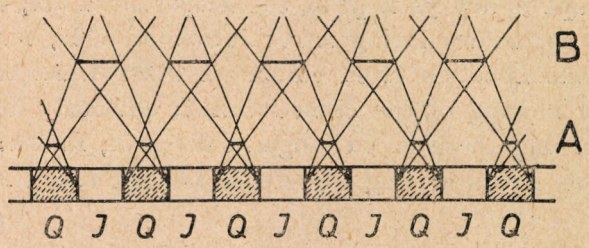


Fig. 3. Explanation of interchange of cross-striation on the basis of *Becke's* phenomenon.

In investigating the significance of this matter, when we wish to observe the shifting of the cross-striation, this circumstance may cause errors, for the fibril in the capillary, because of its excentric position, assumes in turning now a higher and now a lower position (with the

greatest care it is never possible to find fibrils in the capillaries which lie exactly parallel to the capillary axis) and thus the shift of the cross-striation itself can be caused by the difference in distance.

In our experiments we had in any case to make certain that in turning we did not change the distance from the object. We succeeded in this by always examining the fibril in the deeper situation (using the upper-situated ones as controls).

b.) Rotation.

To determine whether the fibril's cross striation moves or not it was necessary to take a fixed basic position to which we could return after the rotation. For this purpose we selected an I stripe beside which small fibril pieces could be seen. We adjusted the cross-thread from this known I stripe to the fourth I stripe without disturbing the particles in the process of the observation.

The rotation was accomplished in such a way that the cross-thread was kept on the I striation during the constant observation of the fibril picture, while the capillary-rotation drum was extremely slowly turned 2°. After each turn, as control, we turned to the higher situation to observe whether the cross-thread stood on the dark stripe. Every 10° photographs were taken.

After a rotation of 360° we returned to the marked basepoint and found the cross-thread on the same I stripe from which we started. (Figure 4.)

DISCUSSION.

The results of the experiment show that if single fibrils of water-beetle's flying muscle are turned round their long axis and the lens of the microscope is always kept at the deeper situation, then the cross striation does not move during a revolution of 360°, but that we return to the same striation from which we started. According to this, observations of moving of the cross-striation obviously derive from the fact that in turning there is a change in distance, BECKE's phenomenon appears and the errors arising therefrom are not taken into consideration.

According this basis, in our experiments at every angle in the revolution we should have got an image of exactly the same character. If we look at the photographs (Figure 4) we can observe consistent changes in the shape and size of the Q and I stripes. In these changes we find regularity if we begin with those photographs in which the I stripes are the largest (at about 130°). At 180° from this point (about 310°) the Q-stripes are the biggest, then in the pictures at 90° from

either, (at about 220° and 40°) the Q and I stripes are equal size. On the basis of observation of the pictures it is our impression that the fibril is not symmetrical but that its front, back, right and left sides could be distingushed and the photographs obtained of this could be the optical demostration of an assymetric structure. It is possible that this refractive assymmetry is caused by the submicroscopic structure of the fibril.

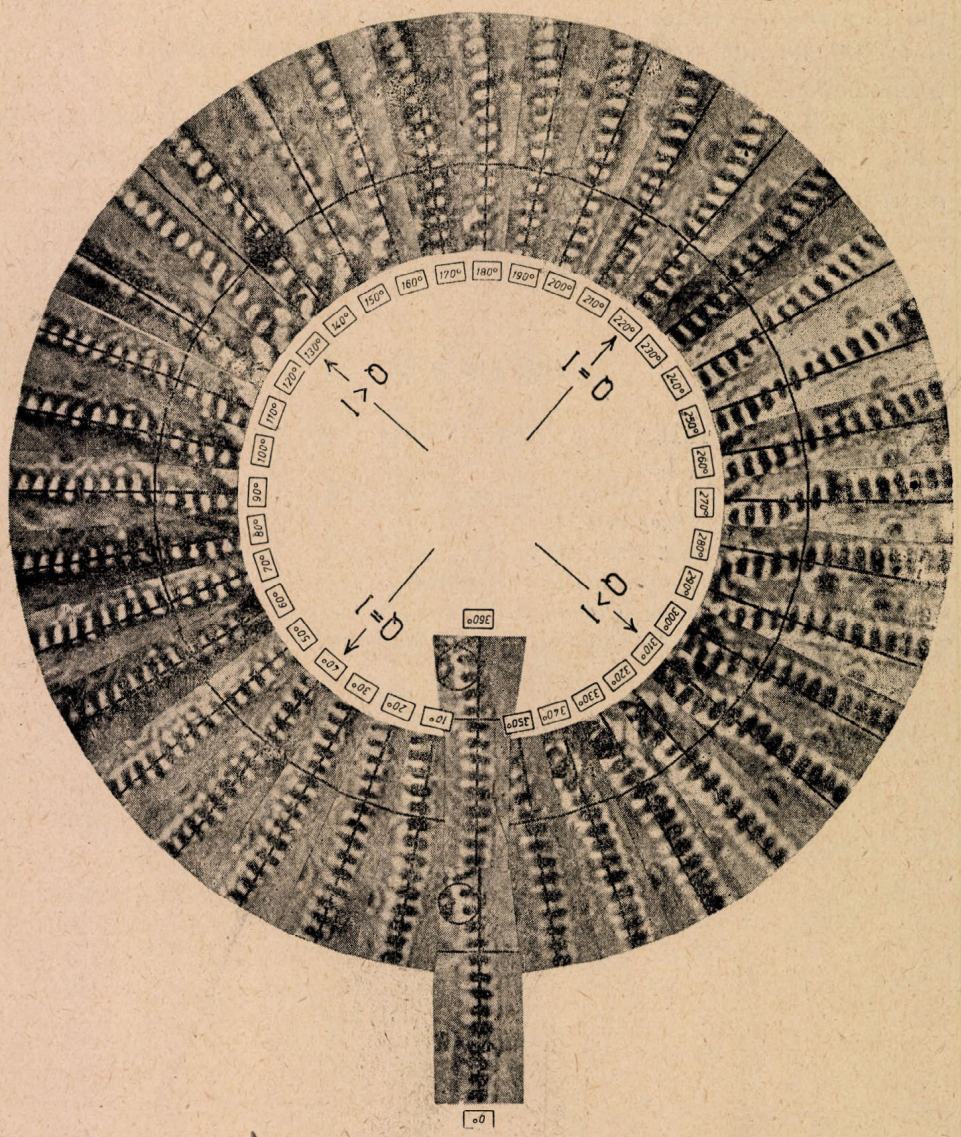


Fig. 4. Microscopic pictures of an isolated wing-muscle fibril of Hydrophilus turned on its longitudinal axis.

SUMMARY.

Microscopic investigation of the fibril of water-beetle's (Hydrophilus) flying muscle established that the cross-striation of the fibrils moves because of changes in distance from the object. Taking this into account, we turned single isolated fibrils identically distant. The cross striation of the fibrils did not move during rotation. According to this the former observations of movement in the cross striation took no account of the differences in distance from the object and of BECKE'S phenomenon.

Photographs taken every 10° of rotation show that the fibril's cross-striation is due to assymmetry in the material's submicroscopic structure.

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