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On muscle fibre

(1858)

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[...] The striated muscle fibre of the fly, taken from various parts of the body or, as is my preference, from the joints of detached legs, consists of a central channel running along its entire length, filled with spherical or oval utricles containing miniscule granules.

The central channel is circumscribed by a kind of sheath composed of a series of flat rings located a certain distance one above the other and held together by a large number of longitudinal threads. These threads are entirely surrounded by a soft utricular tissue.

This utricular tissue is followed by a second sheath organised in a similar way to the first, namely with rings joined together by threads. Lastly, the entire fibre is enveloped by a transparent, crinkled and extremely thin external membrane. The arrangement of the parts I have mentioned can be understood clearly by referring to *fig.* I, which is a cross-section of the fibre magnified 744 times. A is the central channel; B is a ring in the innermost sheath; C is the layer of utricular tissue; D is a ring in the second sheath, similar to the first. The circumference E shows the exterior membrane.

The figure was drawn from life, but it is only with the utmost patience that regular cross-sections can be found. Good fortune is also needed because the cross-sections cannot be obtained at will regardless of the cutting tool that is used; the thinness, softness and contractility of the fibre are obstacles that can be overcome only by performing a multiplicity of trials and at the cost of a great deal of time. But the reality of the matter can be verified quickly and simply by observing the fibre extended lengthwise and using the principles of optics to observe the images that appear.

A muscle fibre extended in the slide can be seen magnified 744 times in *fig.* II. This fibre contains transversal striae, which constitute the profile of the flat rings belonging to the two sheaths B and D in fig. I, and exhibits longitudinal threads that join the aforesaid rings.

Furthermore, if the focus of the objective lies at the midpoint of the thickness of the fibre, within it we can discern three narrow parallel spaces, like three bands M, N, P which appear to divide it into four fibrils. What are these bands? They are the longitudinal sections of the central channel A and of the utricular layer C, as I shall demonstrate. Firstly, if we rotate the fibre around its axis, the positions of the three bands do not change significantly, which means that the middle band represents a cylinder located on the axis of the fibre itself, and the lateral bands derive from the cross-sections of a hollow cylinder concentric with the first. Secondly, if we examine the bands carefully, in the middle one we discover utricles of the same shape and size as those shown in A *fig.* I (further enlarged and separated in T to indicate their granular content) and in the lateral ones we discover the utricles of layer C.

In a further experiment, we focus the objective on the surface of the fibre; in this position the connecting threads and striae can be seen more sharply. The object is then raised with a micrometric movement and new, deeper threads come into focus in place of the former, until we discern the utricular layer which is drawn in a plane in *fig.* III. We again raise the slide by the precise thickness of the utricular layer; this brings other connecting threads into view, after which the central utricles appear. Having reached this point, if we continue to raise the fibre, the images I have described return but in the reverse order. This clearly demonstrates that the fibre is composed of parts organised in different ways and joined together in the manner described above. However, it remains to be explained how the extremely thin membrane enclosing the fibre is distinguished. This membrane can be discerned only when observed very obliquely, and owing to its perfect transparency it reveals no hint of its existence when examined face-on. I first gained an awareness

of it by studying the significance of certain protrusions that project from the walls along the edges of the fibre as shown by F in *fig.* II.

These protrusions are not always visible, and they project to a varying degree depending on the state of tension of the fibre. They are not created by outward folds of the longitudinal threads; and I have been unable to find any explanation other than that they originate from the external membrane, which, adhering only to the circumference of the rings of the sheaths, wrinkles according to the greater or lesser proximity of the rings themselves. This explanation is supported by the observation that if a muscle is left to dry between two glasses, once the contractile substance of the fibre has become opaque and shrunken the external membrane can clearly be seen on its sides. If the two glasses are separated forcibly after drying, on one glass there remains the fibre and on the other the isolated membrane with its clearly pronounced lines; the capillary branches of the tracheae are also visible on the surface of the membrane and never penetrate below it. Another detail of the fibre that can be observed is the speckling of the striae as shown by G in *fig.* II. It appears that every stria and consequently every ring is composed of three flat layers which are joined together to form its thickness. The middle layer is the speckled one, while the other two are smooth and more transparent. It is likely that the speckling originates from the connection of the longitudinal threads that join the rings together.

As I have said, the threads are often folded in a zigzag fashion along the entire length of the fibre, or part of it. This is illustrated in I *fig.* II. It was already known that in the higher animals the fibre has a similarly tortuous arrangement, and whoever has eaten cooked meat, such as beef, will have been able to recognise this without any need for lenses. But if we examine one of these sinuous fibres under the microscope, it will be plain to see that between each fold or corner there are a large number of transversal striae; these are quite different from the zigzag folds described above, which occur between two horizontal striae in the threads.

Without proving the existence of the threads between one ring and another, we would be unable to ascertain the manner in which they fold; and I have reason to believe that the structure of the fibre such as I have long represented it has not come to the attention of the anatomists who have written on this subject.

Each fibre is joined to its corresponding tendon by a hemispherical or round shaped end section. The tendon is subdivided into divergent filaments which are joined to the terminal caps intercepted by the last ring in the fibre (H *fig.* II). In thousands of preparations made with leg muscles, I have only ever found the tendon on one side; I have always observed the other side of the fibre to be free and more or less regularly truncated between two rings. But in the wing I have observed non-lacerated muscles composed of bundles of forty to fifty fibres of length 0‴,35, joined to a tendinous peduncle 0‴,225 subdivided in such a way as to embrace all the terminal caps of the fibres. And on the other end I similarly observed the rounded shape of the fibres joined in the same way to the filaments of the tendon, which instead of extending in a pedunculate tendon, terminate immediately no further away than 0,‴02 and are connected to an opaque cartilage.

Frequently one can see a number of tendinous peduncles carrying a bundle of fibres arranged like an open thistle flower at their free ends. Observing the appearance of the fibres, we can note that the images are not always identical. Sometimes the fibres belonging to a peduncle display the three longitudinal bands in the manner I have described, while in another peduncle the bands of fibres are less distinct and only the central channel can be seen clearly. Lastly, some tendons have fibres which instead of three bands have five, and these are so pronounced that the fibre would be considered divided into six fibrils if a more attentive examination did not reveal this to be an illusion. The appearance of the five bands derives from a second layer of utricular tissue perfectly identical to the tissue of *fig.* III but situated more closely to the circumference of the fibre. This is demonstrated unequivocally by repeating the observations described above to explain the appearance of the three bands. And any attempt to isolate the six assumed fibrils by exerting pressure between the two glasses is bound to fail. All of the fibres I have talked about can only be subdivided by lacerating them.

This resistance does not occur in the softer muscles of the thorax, where the fibrils separate easily when squeezed with moderate pressure between two glass plates. They emerge together with a quantity of globules, or rather utricles, that spread out in water. The fibrils are cylindrical, extremely fine, with a diameter of between one thousandth of a millimetre and 0‴,002. The intermixed utricles have a larger diameter and on average are equal to 0‴,0025. In the normal state, the utricles, which constitute perhaps half the mass of the fibre, have a regular arrangement and form several layers. The longitudinal striae of the muscle are evidently due to the countless multitude of fibrils distributed in parallel bundles. The transversal striae which are revealed at the same time in the muscle, albeit less clearly, originate from the transversal striae which in reality exist in the fibrils of the fly. But without a strongly penetrating optical power it is not possible to discover its true structure. In other insects the fibrils are larger, allowing them to be studied more easily. And because they display a very similar organisation to the fibrils of the striated muscles of higher animals, I shall disclose some observations that I have made in this field, an area in which many capable observers have practised without coming to an agreement, which demonstrates the great difficulty of the subject.

As all anatomists know, it does not take a great deal of skill to isolate the fibre of voluntary muscles. But the fibrils cannot be separated mechanically. To separate them it is necessary to resort to maceration, cooking or the use of chemical reagents. I have adopted various means, but without being entirely satisfied. Nonetheless they were sufficient for my investigations, which convinced me that the fibrils do not consist of a varicose thread, nor of regularly aggregated primitive particles, nor of rectangles or squares joined together in series, nor yet of threads twisted in the manner of a spiral, but rather they are small cylindrical tubes divided transversally by diaphragms in the same fashion in which the rings divide up the fibre of the fly.

Fig. IV shows three lamb muscle fibres magnified by 2000 diameters. The dark cylinders are interposed, approximately at equal heights, by light segments divided into two by a dark line. Using objectives of the highest power with favourable light, one can see dark dotted lines R and dark longitudinally striped cylinders S. The structural similarity between these fibrils and the fibres they derive from is considerable. The only difference is that of their size; because the lamb fibre is nothing other than a repetition of its fibril together with the external membrane that surrounds it. The muscle fibre of the pig and the ox can be decomposed into similar fibrils.

The best drawing of the fibre that I have seen is the one made by Leonard copied from a preparation by Lealand and shown in table 8 *fig.* XI of Quekett’s treatise on the use of the microscope. In his explanation on page 439, the author claims that in the figure magnified 1200 times it is possible to discern that each fibril is composed of bands or stripes of two different alternating structures; and that with more careful examination a transversal line is discovered between each dark band. My observation therefore agrees perfectly with the Englishman’s observation; but the eminent Quekett adds that the transversal line gives the fibril the appearance of being composed of a linear series of square or more or less oblong shaped cells, with a dark substance in the centre of each, and he illustrates this with a diagram, noting that in some cases, as in his *fig.* XI, the dark substance extends to the edges of the cell, preventing it from being seen transparently. In this second part of the observation my opinion diverges from that of the learned microscopist from London. I do not believe that there exist cells containing dark matter filling them to a greater or lesser extent; but rather that the difference between the light and dark parts depends on a difference in refracting power of the alternating segments of the fibrils, which due to their extreme thinness are highly transparent. If a fibril is observed magnified using a good microscope and illuminating the object from below with condensed central lighting, when the edges of the fibril appear most distinctly, the transversal segments can be seen to be alternately light and dark in an identical manner to that shown in my *fig.* IV and Leonard’s eleventh figure. But if from here we proceed to another position by lowering the fibril with micrometric movements, thus moving it away from the focus of the microscope, then a different image appears, namely the dark segments become light and the light segments become dark. If instead of moving the fibril away from the

focus we move it closer to it, in this case the dark spaces become more opaque. The same effect is obtained with a glass cylinder composed of alternating segments of flint-glass and crown-glass, as anyone who has a basic knowledge of optics will not find it hard to understand. I should add that since the diameter of the fibrils is 0''',0016 and the height of the segments is approximately the same, each segment constitutes a very small cylindrical lens which forms the image of the illuminating lens behind it. Of these luminous images, some are smaller and others are larger in alternation, the former deriving from the denser segment and the latter from the less dense segment. What impression will the eye receive on observing a linear series of these indistinctly defined images? It will be that of a transparent thread, alternately thin and thick at equal distances; it will be a *varicose thread*, believed by many, as a result of an illusion, to be the normal type of fibril. [...]

Returning to fibrils from the thorax of insects, which as I said could be studied better elsewhere than in the fly, I shall begin with the wasp. In the thorax of the wasp we find muscle fibres that are striated in both transversal and longitudinal directions and are of greater diameter than the fibres to be found in the legs and wings of the same animal. If large thorax fibres are extracted from a recently dead insect and placed in water, when these fibres are subjected to moderate pressure they dissolve into fibrils varying in diameter from 0''',002 to 0''',004. Examining the largest of these, it is not difficult to recognise their structure, which is identical to that of the fibrils of lamb drawn in *fig.* IV. These consist of very clear small tubes divided at equal distances by diaphragms, which in profile appear to be composed of three layers in contact, the one in the middle speckled, the other two smooth. The segments of the small tubes interposed between two diaphragms clearly consist of a less dense substance because they display the same effects of light refraction that I have described.

The cylindrical shape of the fibrils can be recognised not only by observing them lying down, but is clearly evident when observing their cross-sections, a large number of which are sometimes to be found oriented towards the eye due to the folds they acquire during preparation. The cross-sections are circular and prove that the fibrils are all full; or at least the optical power is not sufficient to reveal any channel or empty space there. Besides the differences in their diameters, which lie between two thousandths of a millimetre and four thousandths of a millimetre, a difference can also be observed in the distances between the segments of the small tubes, which probably depends on the various states of tension. And it is not rare to encounter fibrils which are so taut that their transversal striae are all inclined in such a manner that on first appearance one would consider them to be spirally shaped. The fibrils extend from one end of the fibre to the other without bifurcating or branching; they run parallel in contact with each other in the manner of small bundles. In every bundle the transversal lines are approximately at the same level, but between one bundle and another the transversal lines are not always in contact. Given that the shape of the fibrils is cylindrical, it is understandable that within a bundle the contact between the fibrils cannot be complete; there remain longitudinal spaces that are filled with an extremely fine granular material, which appears to adhere to the edges of some isolated fibrils. The granulations should not be confused with the utricles, a large quantity of which separate from the fibre when squeezed. Internally these utricles are arranged in layers surrounding the bundles of fibrils, and the greater ease of isolating the fibrils is perhaps a consequence of the weakness of their connection.

There are no substantial differences between the wasp and the bee with regard to the structure of the muscle fibre and the dimensions of its parts. Like the fly, these insects can be found alive all year round and can therefore be used to repeat the observations I have described. It would be superfluous to list all the insects in which I have seen the same things; in general beetles are more suitable for investigations of this kind due to the size of their fibres. Using the giant fibres of the stag beetle, I have made many unsuccessful attempts to separate the fibre extracted from the legs into fibrils. I treated the fibre with dilute nitro-muriatic acid and kept it immersed for ten, twenty, thirty days without being able to detach any fibril; in the meantime with the same reagent the thorax fibre was destroyed and reduced to formless granulations.

There is no doubt that the contractile part of the fly fibre extracted from the legs is located between two transversal striae, in other words in the rings that contain the longitudinal threads. This is demonstrated by the observation that when the still palpitating fibre is shortened or lengthened, the thickness of the striae does not change. The striae apparently remain composed of the speckled middle line and the two smooth lateral lines. The variation in distance between them is manifested by changes in width of the intermediate spaces, which are those that contain the longitudinal threads, and precisely those that refract light less, in other words those of lower density. Longitudinal expansion and contraction also occurs in the fibrils although it is not possible to witness the changes as they occur. But it is reasonable to assume that the movement occurs in the same way as in the fibre, and the contractile faculty is associated with the less dense segments S; because in all likelihood perfect similarity of form corresponds to similar functions.

I shall not linger on physiological considerations, but to conclude this work, I believe it opportune to return to the fibril, which, being composed of elements of two different densities, positioned alternately one on top of the other, bears a strong resemblance to the Volta pile.

[*This section of the manuscript was not published*]

Suffice it for me to cite Kölliker, who, while expounding his own numerous observations, does not fail to mention those of others. In his Elements of Human Histology, Paris 1856, p. 223, he publishes drawings of fly fibres extracted from the wing muscles magnified 350 times, which do not show the smallest sign of longitudinal threads connecting the striae. And regarding the question of how the fibrils are shortened and the cause of the transversal striae, he limits himself to two suppositions, either that the fibrils are not homogeneous over their entire length and are divided into a large number of variously elastic small segments, or that they consist of soft filaments which shorten and fold in a zigzag fashion or become undulating or varicose. He concludes that it has not yet been possible to establish which of the two suppositions is true.

Since I have cited a work that is highly esteemed and widely known among us, it is worth commenting here on a difference in nomenclature that may cause some confusion. Kölliker says that the *fibrils* of the thorax muscles of insects are easy to isolate. I believe that it is not the *fibrils* that can easily be isolated but the *fibres*. His *muscle fibrils* of the fly, fig. 110 (synonym for *primitive fibres* defined on page 194), are clearly identical to my fig. 2, which I refer to as *fibre*. This is the first natural division of the muscle, formed from the aggregation of a large number of similar organs. I have not once succeeded in obtaining the subdivisions of the fibre which could appropriately be called fibrils, either through maceration or using chemical reagents. I would be unable to anatomise the fibre of the fly except visually.

(English Translation by John Freeman)