XII. *On the Minute Structure of the Leg-muscles of the Water-beetle. By* Edward Albert Schäfer. *Communicated by* Dr. Sharpey, *V.P.R.S.*

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For the successful prosecution of the histological inquiry which forms the subject of the present communication, it is essential that the tissue to be investigated should be studied whilst still in the living condition, inasmuch as marked changes ensue very speedily after the death of the muscle, and still more speedily on the addition of reagents, even the so-called indifferent fluids, such as serum or $\frac{1}{2}$ per cent. solution of common salt, being in this case inadmissible. The description, therefore, I have now to give is founded entirely on an examination of the living tissue.

I. *Appearance of Living Muscle in the state of rest.*

If we cut off a limb from one of the common large Water-beetles (*Dytiscus marginalis*), remove a portion of muscle from the upper part, quickly separating the fibres somewhat from one another, by means of needles, on a glass slip, cover it without addition, and examine the preparation so obtained with the aid of a very powerful immersion-objective (such as the No. 11 of Hartnack or the No. 3 of Zeiss), we find numerous muscular fibres presenting an appearance similar to that represented in Plate XXXIII. fig. 1.

The well-known transverse bands are conspicuously seen, consisting of broader dim* stripes (*a a*) alternating with narrower bright ones (*b b*), the latter exhibiting a transverse line of minute dots (*c*). These bands are now very generally admitted to represent disks composed of two entirely distinct substances, arranged in successive series with their planes at right angles to the axis of the fibre; and I shall, for convenience of description, adhere for the present to this notion, although, as I shall afterwards endeavour to show, the difference in appearance which they present may be accounted for otherwise than by regarding them as composed of different materials.

To proceed with my description. Each disk of dim substance appears pervaded throughout by a number of excessively fine rod-shaped particles of uniform diameter, and rather darker in appearance than the substance of the disk which they traverse. These particles are arranged closely and very regularly, with their axes in a direction more or less parallel to that of the fibre; they extend at either end into the neighbouring disks of bright substance, becoming somewhat less distinct as they pass

* I use the word "dim" because, in fact, in living muscle at rest no such thing as a *dark* stripe occurs; one part is only somewhat less bright than the other.

into this, and finally terminating near its middle in an enlarged knobbed extremity, which appears as a minute dark dot. Consequently each of the bodies we are now considering, and which from their form may be denominated *muscle-rods**, may be described as consisting of a cylindrical middle part or *shaft*, which is imbedded in the substance of the dim stripe, and of two enlarged extremities or *heads*, which are found near the middle of the bright stripe. It further follows that there are as many series of muscle-rods as disks of dim substance in the fibre. Moreover each rod of any one series corresponds exactly with one in the next following series, their enlarged extremities almost meeting in the middle of the bright substance; this arrangement causes the appearance of a double row of dots (*c*) running transversely across each bright stripe†.

It must be observed, however, that in such a preparation as is here spoken of, the appearance represented in the figure, although very common, is by no means the only one that presents itself. All the variations, however, that are met with are accompanied and, as will be hereafter shown, in all probability caused by variations either in the relative position or in the form of the muscle-rods. Thus we not unfrequently find fibres in which the rod-heads of contiguous series, instead of being in more or less close apposition in the middle of the bright disk, are separated by a distinct bright interval; this is more especially the case when the muscular fibre happens to be somewhat stretched.

Sometimes a greater or less number of the rods of neighbouring series are shifted abruptly upon the rest in a longitudinal direction, so much so that their heads may come to be opposite the middle of the shafts of the remainder (see fig. 1). In such cases the transverse striæ also become correspondingly shifted. This fact would of itself almost preclude the conception of the existence of continuous membranes extending across the fibre, an idea which has been largely prevalent of late years.

The variations which occur in the *form* of the muscle-rods are chiefly, if not entirely, dependent upon the relation in size which subsists between the shafts and the ends, these seeming to be in inverse ratio with one another. Thus the shafts are not unfrequently increased in length, the heads at the same time becoming smaller and closer to those of the next series, so that a double row of dots is no longer seen in the middle

* A word here seems due to explain why the use of the term "sarcous elements"—which was applied by Mr. BOWMAN ("On the Structure and Movements of Voluntary Muscle," Phil. Trans. 1840) to the minute particles of which he conceived a muscular fibre to be composed, and which has been very generally applied by subsequent observers, both here and on the Continent, to the rod-like bodies which are commonly described in the muscles of insects—has been omitted throughout the present communication. The reason is simply this—that the term is ordinarily understood to imply that the bodies to which it is applied form the essential contractile constituent of muscle, whereas it will be seen from what follows that this is probably not the case with the structures here denominated muscle-rods. It should, however, be distinctly understood that the latter term is employed to signify simply the *form*, without reference to the nature of the material of which the particles in question are composed.

† Compare STRICKER, 'Handbuch der Gewebelehre,' p. 1225.

of the bright stripe, having become merged into a single dark transverse streak, having a granular look.

If we imagine this process to go on still further—that is to say, if the shafts go on increasing in size at the expense of the rod-heads until each muscle-rod is of uniform diameter throughout, the ends of the rods of different series being in close apposition, it may well be imagined (the smallness of the objects with which we have to deal being taken into consideration) that it will be extremely difficult to detect the points of junction between the rods of successive series. In this way the continuous longitudinal fibrillation which is often seen in the muscles of the Water-beetle may be accounted for. That each fibril, however, is not in reality continuous throughout the fibre, but consists of a succession of shorter units, the ends of which are in close apposition, is shown, not only by the phenomena which occur when such a muscular fibre is thrown into contraction, and which will be referred to further on, but also by the appearances presented when a portion of the fibre comes under observation in which these units are thrown into zigzag instead of lying in the same straight line, in which case the angles of the zigzag represent the points of junction of the muscle-rods.

It is worthy of note that in all fibres like that we are now considering (that is to say, all in which the rod-heads are of the same diameter as the shaft, so as no longer to appear as distinct objects), the bright transverse bands have entirely disappeared, and the substance between the rods is of a uniform appearance throughout.

II. *Appearance of Contracted Muscle.*

In contracted muscle (fig. 2, C) we observe, besides the general increase in thickness of the fibre and the approximation of the cross striæ, that the latter have become completely altered in character. Dark and light transverse stripes now appear, which are of nearly equal size: the former, instead of being merely dim as in the resting muscle, have a decidedly dark aspect; the light stripes, on the other hand, present a very bright appearance, without any indication of a row of dots, but with sometimes a faint longitudinal streaking.

In order to explain this difference between a fibre at rest and the same fibre in the contracted state, it is necessary to follow all the stages of the process. Owing to the extreme suddenness and rapidity with which the contraction supervenes, and the amount of displacement which immediately precedes and accompanies it, it becomes a matter of exceeding difficulty to follow the details of the process with the high powers which it is necessary to employ. However, it has occasionally happened to me to observe a contraction proceeding at a very much less rapid rate, and in such cases it was possible to make out the following changes:—

The first thing seen is that the bright transverse bands become approximated throughout the contracting portion (P), and with them the dark transverse lines in their middle, which, as previously shown, are made up of a double row of dots (rod-heads). As the contraction continues the rod-heads become larger, and by this means are brought into close relation with the neighbouring ones, so that each double line of dots (c) no longer appears as such but as a single dark transverse band ($c_{\prime}$), which separates the bright stripe ($b_{\prime}$) into two distinct parts. As these dark transverse bands approach one another, their bright borders encroach more and more upon the dim stripe ($a_{\prime}$), until this becomes reduced to a mere line, and finally disappears altogether: so that in contracted (C), as compared with resting muscle, the broad dim stripes (a) are replaced by bright bands ($b_{\prime\prime}$), the clear stripes (b) by dark bands ($c_{\prime\prime}$), these last being the optical sections of the disks which are formed throughout the fibre by the close juxtaposition of the enlarged rod-heads; the shafts of the rods have become much finer than before, so as to be hardly perceptible as they cross the now bright transverse bands.

III. *Transverse Section.*

When a small portion of living muscle is quickly teazed out upon a slip of very thin glass, to which it remains adherent, and the glass is then inverted over a ring of putty on an object-slide, so that the preparation is enclosed in an atmosphere which is saturated with moisture (STRICKER'S moist chamber), we are sometimes fortunate enough, in examining it, to find one or more fibres which are bent upwards near the end, and of which consequently we get a view of the transverse optical section (fig. 3). Employing the No. 11 HARTNACK we observe the following:—

The cross section of the fibre appears of a rounded or somewhat oval form, enclosed by a distinct contour line (section of the sarcolemma), and with a small granular mass in the centre (central protoplasm), which may be almost entirely occupied by one or more nuclei. The area of the section between the sarcolemma and the central protoplasm has a minutely punctuated character, this appearance being due to the presence of a number of minute specks scattered equally over the field; the intervals between them appear homogeneous, clear, and bright. This appearance is obtained to whatever depth in the fibre we focus the microscope. As far as can be made out, these specks correspond with the rods which are seen in longitudinal view. The substance in which they are imbedded is quite similar in aspect to that which composes the bright transverse disks (fig. 1, b); why the dim substance in which, in longitudinal view, the shafts of the rods appear to be imbedded is not seen, as least as such, in the transverse section will become evident when we have treated of the production of the cross striæ.

IV. COHNHEIM'S *Areas.*

If, in order to obtain the transverse view of a muscular fibre, we employ the method recommended by COHNHEIM* (that is to say, if we make a cross section of the frozen muscle and examine it in a so-called indifferent fluid, such as the $\frac{1}{2}$ per cent. solution of salt), we sometimes obtain the appearance described by him of dim polygonal areas, bounded

* "Ueber den feineren Bau der quergestreiften Muskelfaser," VIRCH. Arch. Bd. xxxiv. (1865).

by and enclosed in a network of clear bright lines. It would seem probable, however, that these areas are due to the action of the saline solution; for, as Kölliker* has pointed out, they do not occur if the section is examined fresh without addition. A fine punctuation has been described within Cohnheim's areas†; this is, however, better seen when the limits of the areas are less marked.

It is but seldom that I have been successful in obtaining Cohnheim's areas in sections of *Dytiscus*-muscle. On the other hand, it is not at all uncommon to find fibres the section of which presents the appearance of dark lines, with bright intervals, radiating from the central protoplasm (fig. 4). The lines are equidistant from one another, and now and then divide dichotomously as they proceed outwards towards the sarcolemma. The picture presented irresistibly brings to mind the appearance of a transverse section of an incisor tooth as seen with a low power. The addition of fluid is not necessary for the production of this appearance, as seems to be the case with the areas of Cohnheim.

As regards an explanation of these appearances, I am inclined to think that the section becomes mapped out into areas of Cohnheim owing to a change which takes place in the intermediate substance between the muscle-rods, whereby, instead of being evenly diffused, this tends to collect at definite lines in the fibre, the rods which are enclosed by these becoming on this account more closely grouped, so as eventually to be no longer separately distinguishable.

The radiated appearance above described I was at one time inclined to explain in a similar manner; it may, however, be due to a slight inclination of the muscle-rods towards the axis of the fibre; in this manner they would appear to overlap one another, and so produce the effect of radiating lines.

V. *Ground-substance of Muscle—Cause of the Cross Striæ.*

The muscle-rods do not form by their agglomeration the muscular fibre, but are distinctly seen to be imbedded in a *ground-substance*, the characters of which we must now discuss.

This substance appears, as a rule, in longitudinal view not homogeneous, but, as before mentioned, to consist of alternating disks composed of two distinct materials, of which the one in which the heads of the muscle-rods lie is bright and clear, whereas that which contains their shafts is dim. The fact that the bright transverse bands are often seen to present a slight bulging opposite each of the rod-heads, and that when the latter become of the same diameter as the shaft (so as to be no longer seen as distinct objects) the bright bands also entirely disappear, would seem to show that the bright appearance is an optical effect produced by the presence of the globular heads.

That such an explanation is a possible one is shown by the following experiment:—

A strong solution of gelatine is taken, and having been rendered fluid by heat, a few drops of oil are added and the mixture is thoroughly shaken for a few minutes. By

* "Ueber die Cohnheim'schen Felder der Muskel-Querschnitte," Zeitschr. f. wiss. Zool. Band xvi. (1866).

† Kölliker, *loc. cit.* Engelmann, "Mikroskopische Untersuchungen über die quergestreifte Muskelsubstanz," Pflüger's Archiv, Jan. 1873.

this means the oil becomes suspended in the gelatine in a state of extremely minute subdivision. If, after the mass has congealed, we make a thin section from the middle with a razor, and in the section so obtained examine the minute oil-globules with a high power, they are seen as dark dots each surrounded with a bright halo *. If we happen to come across several such little oil-globules, arranged in a row close to one another, we obtain the appearance of a line of dots in the centre of a bright band.

The bearing of this simple experiment upon the production of the well-known cross striæ of muscle is obvious. For in a muscular fibre there are regularly arranged rows of minute spheroids (the globular rod-heads), the optical effect of which may be con ceived to be quite similar to that of the minute oil-globules in our experiment, viz. to make the substance in which they are imbedded appear brighter in their immediate neighbourhood than it would otherwise be; by this means we obtain the appearance of the bright bands crossing the ground-substance, which have been described by all previous observers, with the exception of HEPPNER† (with whom DÖNITZ‡ would seem to agree in this respect), as constituted by a distinct substance.

It is further to be noted that the neck of each muscle-rod, the part where the head joins the shaft, is comparatively indistinct; this is due to the optical effect produced by the proximity of the globular head, and is contrary to what one would be led to expect if we assume this part of the rod to be imbedded in a clearer substance than the rest, for in such a case it would stand out the darker by the contrast. To this it is probably owing that previous observers have very generally failed in noticing the connexion of the row of dots with the rods.

The appearance of a transverse section is corroborative of the view here taken of the production of the bright bands; for in this case the dots are seen sufficiently close together for the optical effect produced upon the ground-substance by any one of them to be merged into that of the neighbouring ones, so that the intermediate substance appears uniformly bright.

The close relation subsisting between the rows of rod-heads and their bright borders is also seen when a muscle contracts, the movements of the one proceeding *pari passu* with those of the other.

A further indication that the whole of the ground-substance is of the same nature is to be found in the fact that, as will immediately be shown, it exhibits towards polarized light a similar behaviour throughout.

In the state of rest this ground-substance pervades the whole muscular fibre; in con-

* This appearance occurs both when the globules are in focus and also when they are slightly out of the focus; the surrounding brightness may be sometimes seen to be pretty sharply bounded by a margin, which is dimmer than the rest of the field. I am inclined to believe this to be an effect due to the interference between the rays of light which are reflected from the surface of the globule and those which are refracted through its substance. Its bearing upon certain appearances sometimes observed in muscular fibres will be noticed later on.

† "Ueber ein eigenthümliches Verhalten der quergestreiften Muskelfaser," SCHULTZE's Archiv, Band v. (1869).

‡ "Beiträge z. Kenntniss d. quergestr. Muskelf.," REICHERT und DU BOIS-REYMOND's Archiv, 1871.

traction, however, it seems to tend more towards the middle of the muscle-rods between their shafts, thus producing bulgings at the exterior of the fibre opposite these.

VI. *Appearances under Polarized Light.*

Although at first sight it may seem an easy matter to determine which parts of a muscular fibre are singly and which doubly refracting, yet, in fact (owing no doubt principally to the complex optical conditions which are produced by the heterogeneity of the structures through which the light has to pass), there is hardly any subject in histology in which greater difficulty occurs in arriving at a correct result. Accordingly scarcely any two observers who have carefully investigated living muscular fibre with this object have arrived at precisely the same conclusion. One of the chief difficulties is that of obtaining the muscular fibres in a sufficiently isolated state; for the addition of any fluid is quite inadmissible, and without such addition isolation of the individual fibres is liable to be accompanied by desiccation and loss of the normal structural appearances. Of course the presence of other fibres under or over the particular one we are examining may considerably complicate our observations.

Having fully experienced the difficulties of this part of the investigation, I have taken all the more pains to arrive at a trustworthy result, and therefore feel the less diffidence in stating my own conclusions, although they differ materially from those of other observers, with the partial exception perhaps of HEPPNER.

The method adopted has been to make the preparation upon a cover-glass, and to enclose it in a moist chamber in the manner already explained. In this way all possibility of pressure is avoided, and fibres are frequently obtained partially isolated from the rest and perfectly living and contractile. For the purpose of illumination, the oxycalcium light has been employed. For observing the optical conditions, as well as other phenomena, of contracting muscle, I have found it a good plan to pass into the moist chamber (whilst a fibre is under observation) air containing a trace of alcohol vapour. HARTNACK's polarization-apparatus was used throughout.

The opinion, then, that I have been led to form, by a careful comparison of the appearances presented by muscular fibres in the states respectively of rest and of contraction, when placed between crossed Nicols, and with their axes at an angle of about 45° with the principal plane of either prism, may be shortly stated as follows:—Leaving out of consideration the sarcolemma, nuclei, &c., *the whole of the proper substance of a muscular fibre is anisotropous, with the exception of the* structures here designated *muscle-rods, which are isotropous.*

If we observe under the above conditions a living muscular fibre in the state of rest, the whole fibre appears illuminated on the dark field; and in consequence of this illumination we are able to make out the structural points (such, for example, as the presence of muscle-rods) almost as well as when it is illuminated by ordinary light. The rods are clearly distinguishable as dim streaks on the bright ground of the fibre, the heads giving the appearance, by their contiguity, of a single or double dark dotted line. It may be objected that if the rods were isotropous they would stand out as *black* markings on the bright ground: but this does not necessarily follow; for since they are surrounded by, and imbedded in, the anisotropous substance, any rays which reach them from the lower prism must of necessity pass through this substance, and thus be made available for illumination.

In the contracted fibre the conditions are different; for in this case the heads of the isotropous rods are enlarged and in close contiguity, so that there is no perceptible amount of anisotropous ground-substance between them; and this being the case throughout the thickness of the fibre, we get a composite disk of isotropous substance produced, which remains dark between crossed Nicols. On the other hand, the anisotropous substance has now become collected between the shafts of the rods, disks of anisotropous substance being thus formed, which alternate and nearly correspond in size with those of isotropous. Consequently we have the effect produced of a series of illuminated stripes with dark intervals. The isotropous shafts of the muscle-rods, which are present in the former, do not stand out as dark streaks, for the same reason as that just stated in the consideration of the muscular fibre in the state of rest.

HEPPNER* has given a somewhat similar account to that here given of the appearance of muscle under polarized light. He describes a muscular fibre as consisting of only two substances, which he terms respectively *Hauptsubstanz* and *Zwischensubstanz*, which are disposed in alternating disks. Those of the latter substance he considers to be represented by the dark granular line in the centre of the bright bands; all the rest is *Hauptsubstanz*. The latter, according to him, is anisotropous, the former isotropous. Since the granular line referred to obviously corresponds with the line formed by the contiguous heads of the muscle-rods, it will be seen that HEPPNER'S account, so far as the mere description goes, very nearly agrees with that given by me.

An entire corroboration of the statements above given of the appearance of resting muscle under polarized light has turned up quite unexpectedly in a paper by Dr. F. MERKEL†, which has unfortunately only been in my hands a day or two, in which he makes the following statement (p. 294):—

"In a muscular fibre at rest, viewed under crossed Nicols, the contractile substance and the terminal disks appear bright and clear; the intermediate substance on the other hand so delicately (*exquisit*) singly refracting, that, when the field is completely darkened, there is no trace of it to be seen: the doubly refracting parts lie apparently immediately in contact with one another."

Putting aside MERKEL'S peculiar nomenclature of the parts which, according to his view, compose the substance of the muscle, it is clear that the appearance seen by him indicates that the whole fibre remains bright under crossed Nicols.

Further, I may adduce statements of Prof. BRÜCKE, whose well-known views as to the effect of muscular fibres upon polarized light are entirely different from those here

* *Loc. cit.*

† "Der Contractionsvorgang im polarisirten Licht," SCHULTZE'S Archiv, Band ix. (Jan. 1873).

advocated, in support of the accuracy of the description I have given as to the whole of the muscular fibre in a state of rest appearing doubly refracting. BRÜCKE states, in effect, that, in the muscles of water-beetles in the living contractile state, he has but rarely been able to convince himself of the existence of an isotropous substance, that it never is so clearly seen as in dead muscle, but even here may be missed altogether. Very often so little isotropous substance appears as to be only represented in the resting muscle by a row of points, which form coherent stripes only during contraction. BRÜCKE remarks that this condition is probably the normal one during life, since he has most frequently come across it in perfectly fresh living muscle*. It is not difficult to identify BRÜCKE'S isotropous substance, which he also terms *Zwischensubstanz*, with the heads of the isotropous muscle-rods above described. But it may be asked, How comes it that in dead muscular fibres, especially if they have been placed in alcohol and subsequently mounted in Canada balsam or dammar-varnish, even though apparently fully extended, we so often observe broad alternating stripes of anisotropous and isotropous substance? To this I would answer that, although these fibres are not actually shortened, yet the elements composing them have tended to assume the condition that ordinarily accompanies contraction of the fibre (see below). And this opinion is fully confirmed by BRÜCKE'S statement†, that the isotropous disks are always broadest in such fibres as have been prevented from shortening at the time of death. It is, in fact, as if we took a fibre that had become fixed at death in the contracted state and mechanically stretched it out to its original length; in which case we may readily conceive the anisotropous and isotropous disks which are present in that state of the fibre to be equally stretched out.

And here I am tempted to offer a conjecture respecting the probable changes which take place during the contraction of muscle in the disposition of the structures which I have endeavoured to describe.

It is conceivable that the anisotropous ground-substance is the true contractile part of the fibre, and that it is allied in nature to ordinary protoplasm, but without the granular character commonly met with in the latter‡. The muscle-rods may be imagined to be composed of a labile, and at the same time elastic substance of semifluid consistence, possessed of considerable refracting power on light, and in all probability devoid of vital contractility.

* "Untersuchungen über den Bau der Muskelfasern mit Hülfe des polarisirten Lichtes," Wiener Denkschriften, xv. p. 79.

† *Loc. cit.* p. 80.

‡ Since the above was written my attention has been drawn to a paper by M. SCHULTZE (REICH. und DU BOIS-REYMOND'S Archiv, 1861), in which that author gives it as his opinion that the substance between the rod-shaped sarcous elements is the remains of the unaltered protoplasm of the embryonal muscle-cell. He would appear, however, to ascribe to it rather a nutrient than a contractile function, still looking upon these as the active elements of the fibre.

When a portion of muscle is called into activity, the contractile substance, or at least that portion of it which is between the *shafts* of the rods, shortens in the direction of the axis of the fibre, swelling out correspondingly in the lateral direction; the rods consequently become compressed in the centre, so that the substance of which they are composed tends to accumulate towards the ends. But these become also enlarged in another way; for the effect of the shortening of the fibre would be, first to press the heads of those in adjacent series against one another, and then, by a continuation of this process, to force them down upon their own shafts, encroaching upon the substance of these and becoming larger at their expense. The change of form may be most aptly compared to that which happens when the end of a thread of glass is put into a flame, the glass as it softens running up into a spheroid.

When the contraction ceases all the processes are reversed: it is possible that elasticity of the muscle-rods is an agent in restoring the fibre to its original length.

To recapitulate:—The general result of my investigations upon muscular fibre in the living state has been to induce me to regard the less strongly refracting intermediate substance which pervades the whole fibre as the contractile, irritable, and consequently essential part of the muscle, whereas the more refracting substance (which, as a rule, in the leg-muscles of *Dytiscus* appears in the form of distinct rod-shaped particles regularly arranged, but unconnected with one another) is, I think, to be regarded as performing a passive function only, and consequently as unessential to the idea of a muscular fibre, so far as its function of contractility is concerned. It is possible that this substance is analogous to that of which the granules ordinarily found in protoplasm are composed, being, however, in the case of striped muscle, for some reason at present unknown to us, arranged in a definite manner. That the ground-substance of muscle is doubly refracting, whereas ordinary protoplasm, such as that of which the pale corpuscles of the blood are composed, is not, cannot be taken as a proof of dissimilarity in nature; for, as is well known, the substance of which the cells of plain muscular fibre is made up (the protoplasmic nature of which few, I imagine, would be prepared to deny) is also doubly refracting. And since, according to the view here adopted, the more refracting substance is to be regarded as the non-essential part of the fibre, we must not be surprised if differences occur in the mode of its arrangement in the muscles of different animals, or even in different muscles of the same animal. In other words, it does not seem imperative that a typical structure should be selected to which all striped muscles must necessarily exactly conform; but, on the contrary, differences in the arrangement of the non-essential elements may, and undoubtedly do, occur without corresponding differences in the essential functional activity of the fibres.

A great deal has been written within the last few years with regard to the structure of striped muscular fibre. Previously to 1868 the views of BOWMAN, as modified by ROLLETT, were very generally accepted as affording an explanation of most of the appearances observed; some histologists, however, *e. g.* KÖLLIKER, were disposed to regard fibrils extending from one end of the fibre to the other as the ultimate elements of the muscle. In the year mentioned, KRAUSE, in a paper on the subject*, drew particular attention to the transverse line in the centre of the bright band, and, looking upon this as the optical section of a fine membrane stretched transversely across the fibre, described the latter as being divided into a number of flat compartments (*Muskelfächer*), each containing a disk of solid substance in the centre, separated from the membrane above and below by fluid. At the same time, taking the fine lines bounding the polygonal areas described by COHNHEIM in transverse sections to be the optical section of similar membranes running parallel with the axis of the fibre, and of course meeting the others perpendicularly, he described the flat compartments as thus subdivided into a number of muscle-boxes (*Muskelkästchen*), each of them bounded by membranes both at the ends (bases) and sides, each base-membrane being common to two contiguous muscle-boxes. Each muscle-box, he considered, is almost filled by a portion of the dim substance (muscle-prism), this being separated from the base-membranes by fluid. Subsequently KRAUSE described the muscle-prisms as consisting each of a bundle of more minute particles, which he termed muscle-rods (*Muskelstäbchen*). [The transverse line bisecting the clear stripe was known long previously to KRAUSE's paper on the subject. It was described by BUSK and HUXLEY† as occurring in the muscles of insects; and since they observed with marvellous correctness that it was not so much a continuous line as a succession of contiguous dots, it was supposed by them to be due to the existence of a row of minute sarcous elements interpolated between the rows of larger ones, which were regarded as forming the darker stripes of the fibre. The rod-like character of the so-called sarcous elements had also been long known: it was distinctly figured by BRÜCKE; and SHARPEY had more lately drawn attention to the close connexion which exists between these rod-shaped particles and the minute dots composing the transverse line, only just falling short of tracing the one into the other—this, too, both in the muscles of insects and in human muscle‡.] HENSEN §, writing about the same time as KRAUSE, gave a somewhat similar account of the structure of muscle, also drawing especial attention to the apparent membrane bisecting the light stripe; as regards some points, however, his account was entirely at variance with that given by KRAUSE.

The next contribution to the subject of any importance was that of HEPPNER‖, who employed the still living contractile fibres of *Hydrophilus piceus*. He assumed the dark transverse line in the middle of the clear stripe to represent a *continuous disk* composed of a substance (*Zwischensubstanz*) possessing a *lower* index of refraction than the rest of the fibre, and endeavoured to show, by the assistance of a diagram, that the bright

* "Ueber den Bau der quergestreiften Muskelfaser," Zeitschr. f. rat. Med. xxiii.

† KÖLLIKER's 'Manual of Human Histology.' Sydenham Society's Translation, 1853.

‡ QUAIN's 'Anatomy,' 7th ed. 1867, vol. i. p. cxix.

§ Arbeiten aus dem Kieler physiologischen Institut, 1868.

‖ "Ueber ein eigenthümliches optisches Verhalten der quergestreiften Muskelfaser," SCHULTZE's Archiv, v. 1869.

appearance on each side of it is produced by a total reflection of the rays of light from its surfaces—considering, from the fact of its similar behaviour under crossed Nicols, the substance forming the clear stripe to be similar in nature to the rest of the muscle-substance, with the exception of the *Zwischensubstanz* before mentioned. As a further indication that the bright bands are due to such an optical effect as he described, HEPPNER remarked that their position became altered according as the mirror was inclined or the stage rotated.

Quite recently three important contributions to the study of the structure of striped muscular fibre have appeared; those, namely, by FLÖGEL*, MERKEL†, and ENGELMANN‡ respectively. MERKEL, studying the fibres of the Arthropoda, principally by means of alcohol preparations, finds the transverse line of KRAUSE to be not simple, as believed by the latter, but in reality to be made up of two lines representing juxtaposed membranes (terminal disks or *Endscheiben*), best seen when the fibre is stretched. He further describes an exceedingly indistinct disk (*Mittelscheibe*) in the middle of the dim stripe of the muscle, not always visible, and often only represented by a somewhat lighter appearance of that stripe at this part. The contractile substance of the muscle he describes as being, in the state of rest, arranged on either side of this median disk, with a clear fluid separating it from the terminal disks above and below. The enclosure of each muscle-element is completed at the sides by a lateral membrane. In contraction of the muscle MERKEL describes the contractile substance as at first becoming diffused throughout the muscle-element, and finally becoming collected in the vicinity of the terminal disks. By what mechanism such locomotion is capable of producing shortening of the muscle is not explained§.

FLÖGEL, who for the most part employed muscle of *Trombidium* which had been treated with osmic acid, describes a fibre as divided into compartments, similar to those described by KRAUSE, by means of transverse partitions (*Querwände*), which, however, he believes to be formed by the juxtaposition of granules interpolated at regular intervals in the course of the fibrils, which latter extend from end to end of the fibre, imbedded in a ground-substance which is probably of a fluid nature. In the median portion of each muscle-compartment these fibrils, if I understand FLÖGEL's meaning aright, are more marked (*Säulen*) than near the transverse partitions, in the immediate neighbourhood of which the substance is clear and homogeneous, but contains both above and below each transverse partition a row of minute granules (granule-layer); and since it may sometimes be seen that each granule lies in the same longitudinal line as one of the minute columns in the central part, the two are probably parts of the same fibril.

* "Ueber die quergestr. Muskeln der Milben," SCHULTZE's Archiv, viii. Nov. 1871.

† "Der quergestreifte Muskel," SCHULTZE's Archiv, viii. Jan. 1872.

‡ "Mikroskop. Untersuchungen über die quergestr. Muskelsubstanz," PFLÜGER's Archiv, vii. Jan. 1873.

§ It is but fair to add that MERKEL's results are principally based upon observations on the so-called fibrils of the thorax of insects; and these present in many respects a structure entirely different from that met with in the muscles of the legs, this apparent difference being due to a different arrangement of the non-essential substance (compare p. 438).

A view of muscular structure still more complicated has very lately been set forth by ENGELMANN in his exceedingly elaborate article on the subject. ENGELMANN enumerates no less than ten distinct layers as included in each muscle-segment or compartment (*Muskelfach* of KRAUSE). Each of the segments, according to him, is bounded at each end by a thin but well-marked layer (*Zwischenscheibe*), often appearing granular, and which is common to two contiguous segments. This corresponds with the transverse partition of FLÖGEL. Passing inwards from these we come first upon a clear layer, then a somewhat dark layer (*Nebenscheibe*), which often appears to consist of a row of granules (the granule-layer of FLÖGEL), then upon another clear layer, and finally, in the centre of the muscle-compartment, a broad dim layer (*Querscheibe*), the middle portion of which appears somewhat less dim than the rest (*Mittelscheibe*). Each of these layers is described with great minuteness by ENGELMANN. He does not, however, maintain that they are all to be made out in every case, but only in those instances in which a considerable interval separates the stripes. He further asserts that the *transverse* markings are the only ones which are met with in the living fibre in its normal condition; and in proof of this he states that the transverse disks appear completely homogeneous when viewed *in situ* in the living animal (as, for instance, in minute transparent Crustacea), exhibiting, therefore, no trace of longitudinal striation. The pallisade-like arrangement of the elements which has been described is, he maintains, due to a change which occurs at the moment of death of the fibre, those portions of a fibre in which individual rods have become visible being, according to him, no longer irritable. To explain these so frequent appearances, ENGELMANN assumes the muscular substance to be wholly composed of closely compressed, elongate, prismatic elements extending the whole length of the fibre, and exhibiting at regular intervals differences in chemical and physical properties, producing thus the several transverse layers. He conceives that at the moment of death of the fibre a coagulation and shrinking of these elements take place, accompanied by expression of fluid; and in this way accounts for the fibrillar and granular character which the transverse disks commonly exhibit.

Of the accounts given by the various authors with regard to the behaviour of muscle under polarized light, I have thought it best to say nothing in this place—partly on account of the irreconcilable differences of opinion on the subject, partly because the question has already been very fully discussed in a preceding page.

It only remains for me briefly to point out the parts which, in the several accounts here quoted, seem to correspond with those enumerated by me in describing the appearance of the living muscle of *Dytiscus*. But, first, I would take the opportunity of expressing my unqualified dissent from the positive assertions of ENGELMANN, above noticed, with regard to the homogeneous appearance of muscular fibre in the living state, at all events so far as *Dytiscus*-muscle is concerned. I would affirm, on the contrary, that *however quickly* the preparation is made (and the quicker the better), we find numerous muscular fibres exhibiting the structure I have described, in which the muscle-rods appear with perfect distinctness throughout the whole extent of the fibre, and that such fibres are capable of repeatedly undergoing the most complete spontaneous contraction: indeed after the death of the fibre the distinctness of the picture becomes always very much impaired, and often the structural appearances become entirely obliterated. Further, I may remark that since reading ENGELMANN'S article I have taken what opportunities offered of examining the muscles of minute aquatic Crustacea and insects whilst still in the living body, and as a result of such examination may state that, in spite of the difficulties to observation offered by the presence of the chitinous integument and the small size of the muscular elements, there is no great difficulty, with a good immersion-glass, in seeing that they possess an apparent longitudinal fibrillation. If further proof were needed, it is to be found in an exceedingly interesting paper by Professor WAGENER*, in which the appearances observable in the muscles of the *Corethra*-larva in the living state are fully detailed. But to return to our subject.

The transverse membrane of KRAUSE and the narrow disk of intermediate substance (*Zwischensubstanz*) of HEPPNER would seem to be referable to the heads of two contiguous series of muscle-rods, which, as before stated, often meet in the middle of the clear stripe. The terminal disks of MERKEL, the granule-layers of FLÖGEL, and the *Nebenscheiben* of ENGELMANN are also, I think, to be referred to the same structures, which are by these observers correctly differentiated into two rows. The *Stäbchen* of KRAUSE and ENGELMANN and the *Säulen* of FLÖGEL would appear to correspond with the shafts of the muscle-rods of my description. There remain the *Mittelscheibe* of MERKEL and others, and the line between each two muscle-series, which is to be seen only in very extended conditions of the fibre (*Querwand*, FLÖGEL; *Zwischenscheibe*, ENGELMANN). To explain these appearances, it is necessary to revert to what has been said with regard to the minute oil-globules in the experiment before mentioned†. It was there noted that the bright halo around each oil-globule is often seen to be bounded by a marginal line rather darker than the rest of the field, and probably to be explained by interference. The granules in muscle (rod-heads) must each have a similar effect upon the light; and since they are disposed in rows, so that their bright haloes coalesce into bright bands, these darker margins must similarly coalesce into dim bands. Consequently the edges of the broad dim stripe (*Querscheibe*, Plate XXXIII. fig. 1, *a*) of muscle will be caused to appear somewhat darker ‡ than the central part (*e*), which latter has been for this reason taken for a distinct layer (*Mittelscheibe*). Of course in the case of a muscular fibre all these effects are very much more marked than if we had to deal with a single series of granules, since other rows which are somewhat out of focus contribute to increase the result §.

* "Ueber einige Erscheinungen an den Muskeln lebender Thiere," Sitzungsb. d. Gesellsch. z. Beförderung der gesammten Naturwissenschaften zu Marburg, No. 8, August 1872.

† See footnote, page 434.

‡ The contrast with the bright band would probably tend to enhance this effect.

§ When a very strong light is employed coloured fringes are often visible on the confines of the dim and clear stripes, and doubtless result from such interference effects as I have indicated.

The *Querwand* or *Zwischenscheibe* may probably be similarly explained; for where it appears the rows of rod-heads of adjacent series are so drawn away from one another by the extension of the fibre that their bright borders are no longer blended, and a dim line (in all probability rendered darker by interference) comes into view between them. The argument in favour of the existence of a continuous membrane across the fibre, drawn from the fact that indentations of the sarcolemma pretty constantly occur at this place, loses its weight when we reflect upon the extreme delicacy of the sarcolemma, and the necessity which must exist for it to follow any changes of shape which may occur in the enclosed substance.

Description of the Plate.

PLATE XXXIII.

Fig. 1. Portion of a muscular fibre of *Dytiscus* in the state of rest.

a a a. Dim stripes.

b b b. Bright stripes.

c. Double dotted line in bright stripe formed by the heads of

d. The muscle-rods.

Fig. 2. Muscular fibre undergoing contraction.

R R. Portions still at rest.

P P. Contraction proceeding.

C. Contracted portion.

$a\ a_{\prime}$. Dim stripes.

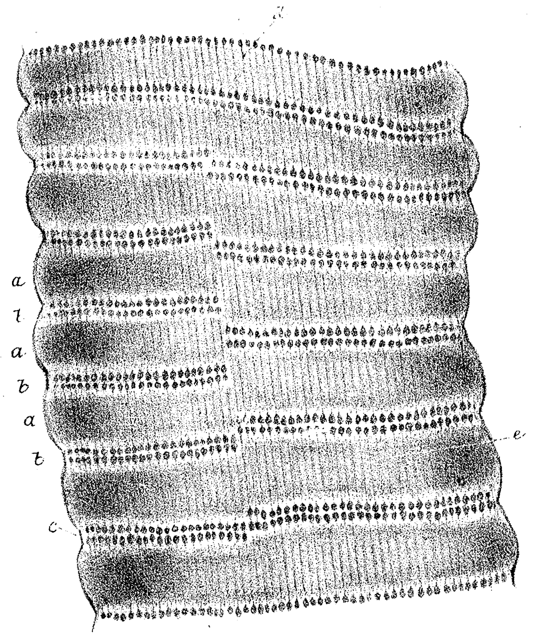
$b\ b_{\prime}\ b_{\prime\prime}$. Bright stripes.

$c\ c_{\prime}\ c_{\prime\prime}$. Lines formed by rod-heads.

Fig. 3. Transverse section of muscular fibre of *Dytiscus.* The irregular patch in the centre, containing a round nucleus, is the so-called central protoplasm.

Fig. 4. Transverse section of muscular fibre of Water-beetle, made whilst frozen, showing the appearance of radiating lines described in the text.

1



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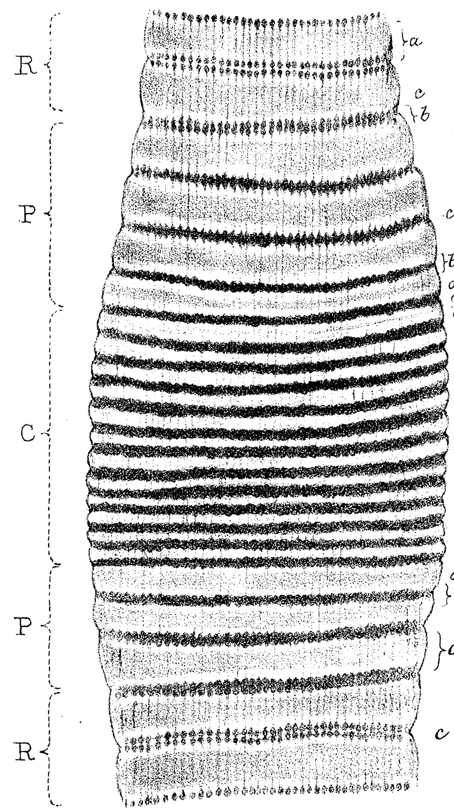

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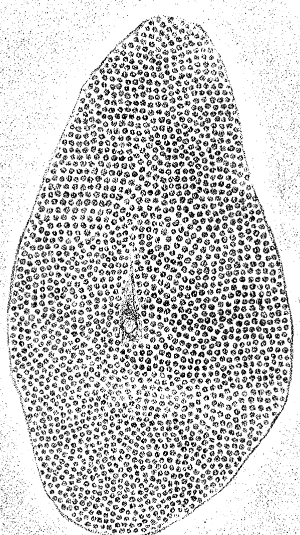

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