February 3, 1881.

THE PRESIDENT in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The Right Hon. Mountstuart Elphinstone Grant Duff, whose certificate had been suspended as required by the Statutes, was balloted for and elected a Fellow of the Society.

The following Papers were read:—

I. "Upon the Cause of the Striation of Voluntary Muscular Tissue." By JOHN BERRY HAYCRAFT, M.B., B.Sc., F.R.S.E., Senior Physiological Demonstrator in the University of Edinburgh. Communicated by Dr. KLEIN, F.R.S. Received December 1, 1880.

[PLATE 5.]

The structure of striated muscular tissue has occupied the attention of many histologists, and various, often antagonistic, have been the views held from time to time since Schwann first investigated this difficult subject.

I bring forward with much caution and hesitation any opinions of my own, nor should I venture thus far, did I not consider my views susceptible of direct proof, or disproof, not being matters of mere speculation, which may or may not be true, and which would tend, by their introduction to the literature of the subject, to make confusion worse confounded.

In this paper an attempt will be made to account for many of the observed structural phenomena of muscle on simple laws of geometrical optics, which will, if it be successful, reduce the subject to comparative simplicity. I shall commence by giving a sketch of the views of those physiologists who have especially written upon the structure of muscle. This must not be looked upon as a complete history, for I shall leave out entirely points which do not concern us here.

A Short Historical Sketch of the Views held upon the Structure of Striated Muscle.—The writings of Mr. Bowman form the most im-

portant and brilliant contributions to the literature of this subject, and taking him as a landmark, it is convenient to speak of investigators before or after his time. Among the former Schwann, quoted by Müller ("Physiology," translation by Baly, vol. ii, p. 878), describes the striated voluntary fibre, indicating its shape and size. The cross markings were observed by him, and, indeed, with one or two remarkable exceptions, by all the early observers (Lauth and Wagner, in Müller's "Archiv für Anatomie und Physiologie, und Wissenschaftliche Medicin," pp. 4 and 318, of the year 1835). Schwann, with Bauer, Krause, Müller, Home, Valentin, and Milne Edwards recognised the important fact that each fibre is composed of a number of threads or fibrillæ, packed side by side and joined together by a transparent tenacious fluid (Krause), and, moreover, that these threads or fibrillæ are cross striated, as is the fibre itself. Although Schultze describes the fibrillæ as being uniform filaments, he is alone in this opinion, most of his contemporaries recognising the beaded appearance.* The beaded thread was the cause of some dispute, for the question arose, was it a linear series of globules or a moniliform filament? and the final settlement of this must, indeed, have been a matter of great difficulty to those older *savants*, when we consider the imperfect lenses at their disposal. Krause and others maintained the former view, while Schwann held that which subsequent investigators have shown to be the correct one. The fibrillæ, according to Schwann, present a very regular succession of bead-like enlargements, darker than the very short constrictions which lie between. Thus, before the time of Mr. Bowman, the following important facts had been made out, namely, that the fibre is composed of a bundle of beaded fibrillæ cemented together, and that the fibrillæ are cross striped, giving the whole fibre a like appearance of striation. Erroneous views had often, it is true, been advanced, but these had never received general acknowledgment. Mr. Skey ("Phil. Trans.," 1837), for instance, considered the fibres to be tubes filled with a soluble gluten, the striæ surrounding and binding them together. Leeuwenhoek had a somewhat similar view of the construction of the cross striæ, and Prochaska considered them as depressions caused by the clasping of neighbouring capillaries and thready tissues.

Mr. Bowman communicated to the Royal Society, in 1840, a paper "On the Structure and Movements of Voluntary Muscle," in which he confirmed many of the opinions of his predecessors, adding, at the same time, much of what was fresh to our store of knowledge. He it was who first described the thin elastic membrane (sarcolemma) covering and ensheathing the fibres, showing how easily to demon-

* Consult a drawing by Allen Thomson in illustration of Dr. Martin Barry's paper on the structure of muscular fibrils, "Phil. Mag.," series 4, vol. 5, Plate V, fig. 2.

strate its existence, and giving figures of it, which have been copied into most modern histological works. The nuclei of the sarcolemma he also figured, but what most concerns us is his description of the cross striation. Bowman, I believe, first pointed out that not only can a fibre be split up longitudinally into fibrillæ along certain dark lines which may generally be seen, even in fresh preparations, but that it splits up transversely along the dark stripes. Each fibrilla may, therefore, be split up into tiny segments across the dark striæ. "On the whole, little doubt remains in my mind that the fibrillæ consist of a succession of solid segments or beads connected by intervals generally narrower, and I believe the beads to be light, and the intervals the dark spaces when the fibrilla is in exact focus." His idea of a fibre naturally follows from that just given of a fibrilla, and, quoting again from him, we find "a fibre consists of sarcous elements (so he termed the little segments or beads) arranged and united together endways and sideways, so as to constitute in these directions respectively fibrillæ and discs, either of which may in certain cases be detached as such," and "the dark longitudinal striæ are shadows between fibrillæ, the dark transverse striæ shadows between discs."

It will be seen that in one particular Bowman disagreed with Schwann and the older writers, and at the same time with those of more recent date. According to him, the bead was light and the constriction dark, when the muscle was in exact focus, a description at variance with everyone. In the same paper he mentions this remarkable fact, that on altering the focus the stripes were reversed; he must have examined it—this bears in a most important way on our investigations, to be afterwards described—in the reverse focus of what it is ordinarily figured in. His view of the form, and the splitting of the fibre, was probably correct, for he described the cleavage as occurring in the narrow part, which appeared to him, focussing as he did, to be dark, and indeed it is often difficult to say which it is, whether dark or light, for, as I shall more particularly mention afterwards, the slightest alteration of the focus is sufficient to reverse the appearance of the fibre. Bowman, moreover, accounted for these light and dark parts of the fibrillæ, comparing a muscular filament to a glass rod with alternate swellings and depressions, which, when viewed with transmitted light, gives just the same appearance, and from a study of his paper, although it is here somewhat indefinite, I judge that he concluded the moniliform shape to be a cause of the striping.*

* Bowman, nevertheless, seems to consider the dark stripe of a different structure from the light, not so much from the shading, but from the transverse cleavage. He is not quite definite here, but this is the impression I have gained from a careful perusal of his paper.

Now, this last-named and important discovery of Bowman's has, I believe, completely been lost sight of, for no mention of it can be found in any modern monograph nor in any systematic text-book that I have examined. The striking points in the paper and in the figures he gives, is the splitting up of the fibre into transverse discs and the demonstration of the sarcous elements as before quoted. This, together with the sarcolemma, everyone connects with the name of Bowman. Modern investigators have worked mostly at the cross striping of muscle, and have found it more complicated than Bowman described, owing, no doubt, to the use of better glasses; while he explained the phenomenon as due simply to the shape of the fibres—believing, however, probably that it was due also to structural differences—modern investigators have introduced hypotheses to account for it, which imply differences of structure along the filament. The reason of this is, if I may express an opinion, that his theory has been completely lost sight of, and that it was followed by the discovery of startling facts, which at first sight seemed to set it on one side.

In discussing the views of modern inquirers, I shall not, in all cases, consider them in the order of their priority, and allusion will not be made to much that has been written upon this subject, which, indeed, may safely be put on one side.

The light stripe—dark stripe of Bowman—has been shown by Dobie, Busk, and Huxley to be traversed by a very fine dark band, or rather line, "Querlinie," dividing it into two equal parts. We shall speak of this as Dobie's line, or the dark stripe in the centre of the light. (Fig 1, D, Plate 5.) Then, again, the dark stripe is traversed in its centre by a lighter band called Hensen's stripe.* (Fig. 1, H, Plate 5.) Other bands border this stripe, but as they are certainly not to be seen in all specimens however well prepared, and as we shall presently account for them, they need not trouble us here.

As early as the year 1839, Boeck showed that muscle refracts light doubly, which statement was, however, modified in 1857 by Brücke. The latter examined muscles prepared in alcohol by polarised light, and found that the dark stripe (dark in ordinarily non-polarised light) appeared luminous in the dark field of the microscope, and that the light stripes were dark when the Nicols were crossed. The dark stripes, therefore, appeared to be doubly refracting (anisotropous), and the light stripe singly refracting (isotropous), the fibre consisting of singly and doubly refracting discs alternating one with another. These observations he verified by an examination of the fibre with thin plates of selenite and mica. The views of Brücke have, in their turn, received considerable modifications which will be understood by reference to a diagram. Fig. 2, Plate 5, expresses

* This stripe was also described by Dobie in the "Annals of Natural History" for 1849, and it may be called "Dobie's light stripe."

very well the results of my own observations, which I find, are in accordance with those of other observers. (See the "Handbuch der Physiologie," by Dr. H. L. Hermann, 1879, p. 20.) The black part of the diagram corresponds with the portion of the muscle which singly refracts light (isotropous), while the light shaded parts correspond with the anisotropous substance.

This diagram does not, it will at once be seen, correspond with the views held by Brücke, for the great mass of the light stripe, with Dobie's line in the centre of it is anisotropous, the dark band, as with Brücke, being anisotropous. The most recent view is, then, that both the light and the dark stripes doubly refract light, but that there are bands which lie between them and which are singly refracting. The appearance which partially warrants such a conclusion I have observed, but I shall endeavour to show hereafter how this may most satisfactorily be explained. It will readily be seen how Brücke's view, until quite recently accepted, would drive one to the conclusion that the light and dark stripes represent two different structures alternating in the length of the fibre, and this is corroborated by statements as to the action of staining agents on the tissues.

Muscle is readily stained by picric acid, but is but faintly tinted by carmine, logwood, or eosine, although Ranvier, in his "Traité Technique d'Histologie," states that he has obtained very beautifully stained preparations of insects' muscle, when using Bœhmer's solution of logwood. According to this observer, the dark stripes as well as Dobie's lines are stained, while the rest of the fibre remains colourless. Klein in his "Atlas of Histology" figures the sarcous matter of the dark band clearly tinted, while that of the light stripe is absolutely colourless. The statement will not be far wrong, that everyone at the present time considers the dark and light stripes as representing two different structures, distinct one from another in their physical properties, for the dark stripe is spoken of as possessing a higher refracting power than the light, and chemically, for their compositions have already been hinted at by more than one observer. The dark stripe is looked upon by most as the true contracting part of the fibre, and they are termed the sarcous discs, or "Muskelprismen," "Hauptsubstanz," or masses of "disdiaclasts," and the light stripes as merely connecting matter, "zwischensubstanz," or "Muskelkästchen flüssigkeit." Dobie's line—more especially from the dipping down and attachment of the sarcolemma in insects' muscle at this point—has been looked upon (Krause, "Allgemeine und Mikroscopische Anatomie," section Muskel System, pp. 80—90) as a delicate transverse membrane. This view has received the assent of such microscopists as Klein and Ranvier, but not of Wagener ("Jahresberichte der Anatomie und Physiologie," Hofmann and Schwalbe) and

Rutherford ("Text-book of Physiology," p. 128), who describe Dobie's line as consisting of a row of dots. Engelmann, indeed, describes a row of dots on either side of this line.

Krause would have us believe that the fibre is divided by these membranes into a linear series of little boxes, each box or casket, "Müskelkästchen," containing a dark stripe with (as the membrane lies in the centre of the light stripe) one half of that on either side. Merkel ("Lehrbuch der Gewebelehre," Stuttgart, 1877, p. 83), to make the "Müskelkästchen" self-containing, affirms that the membrane of Krause is double. As to the stripe of Hensen, this is by very many looked upon as still another structure lying in the centre of the dark stripe; it is in many fibres very clearly to be made out, its border being well defined, and in stained preparations (logwood) it has decidedly a lighter tint than the rest of the stripe. Still some (Krause) look upon it as an indication of the highly refracting power of the dark stripe, comparing the appearance with the light centre of an oil globule. The other cross striæ, of which there are many described by some observers, but none at all universally accepted, are, as a rule, considered as indicating further complications in the muscle fibre; indeed, the "Müskelkästchen," by most advanced microscopists, although not $\frac{1}{10000}$ of an inch in length, consists of some ten or twelve different parts. We may postpone, I think, indefinitely the consideration of these details.

While there is great unity as to the appearance of a fibre during a state of rest, the changes which the fibre undergoes when passing into the contracted condition are not at all understood. Not only does one fail to find among histologists agreement as to the changes in appearance, but the interpretations of these are as numerous as the investigators themselves. All are agreed, that during contraction, the fibre as a whole shortens and thickens, but the changes in form which the cross striæ undergo are not understood so well.

Klein, in his "Atlas of Histology," maintains the broadening of both stripes transversely, the dark stripe becoming thinner in the long axis, and the bright stripe more opaque. Ranvier ("Traité Technique d'Histologie," p. 489) states that the only points one can conscientiously observe in the contraction of a living fibre are, that a knot or bulging forms, in which the dark bands approximate, being only separated by Dobie's line. This led him to believe that the dark bands are the true contracting part of the fibre. Ranvier worked especially with osmic acid, fixing the fibres when at rest, and during contraction. W. Krause ("Allgemeine und Mikroscopische Anatomie," p. 92) describes the contraction as follows:—The thickness (in the length of the fibre) of the dark stripe or an isotropous substance remains the same as far as can be seen, while the thickness of the isotropous substance, "Zwischensubstanz" becomes less. From this, he argues that

the substance of the clear stripe, which he considers as fluid "Muskelkästchenflüssigkeit," passes between the little elements of the dark stripe, causing their lateral separation, and therefore broadening and shortening the fibre. Engelmann ("Neue Untersuchungen über die Microskopischen Vorgänge bei der Muskelcontraction," in "Pfluger's Archiv," Band xviii) is certain that the light stripe during complete contraction becomes darker than the dark stripe, and that there is a period as naturally follows from this observation, when the fibre is quite unstriated. The stripes are in fact reversed, the bright one becoming the darker, and *vice versâ*. Both stripes narrow, but especially the bright one. Engelmann advances a theory to account for this, holding that the cause of contraction is the passage of fluid from the isotropous clear stripe into the anisotropous substance; the former shrinks, and the latter swells. Most startling is the view of Merkel ("Hofmann und Schwalbe," vol. i, p. 116), who believes that the dark stripe shifts its position, arranging itself by Dobie's line, while the light stripe passes to the centre.

It is, as will readily be admitted, somewhat difficult to know what to believe, for there is such entire disagreement among physiologists as to simple facts, to say nothing of any conclusions which may be drawn from them. Thinking that there must be some simple clue which would solve the whole problem, I commenced to work at the subject in the summer of 1878. At the onset the clue was discovered, and the substance of the present paper was written by the end of that year, before I had read for the first time the paper of Mr. Bowman's, in the "Transactions" of this Society. My astonishment was indeed great to find in it the first glimmerings of my own opinions, for although the subject had then been worked out but in the rough, and Mr. Bowman had a much simpler problem to deal with, yet undoubtedly he held the same views in the main. My obvious course was therefore entirely to re-write my paper, making every acknowledgment to his already published work. He considered, as far as I can make out, that the light stripe was to be compared with the cement seen in longitudinal fibrillation, between the fibrillæ, yet he looked upon the striæ as being due to the shape of the fibre. From the history of the subject, which has just been given, it will be seen that all observers are not agreed as to the actual appearances of a striped fibre, and especially the changes which occur during contraction, and I hold that they have fallen into great and unwarrantable error in the conclusions (these, indeed, are all contradictory) drawn from these appearances. A fibre has been observed in the field of the microscope, which is marked transversely, as already described, and all modern investigators have concluded that the transverse bands mark the positions of disks (seen on edge) of tissue of different refractive indices and chemical composition, alternating in the long axis of

the fibre. This is, however, purely an assumption which in no way follows.

We can also account for all these cross markings in a way which involves no theory, and requires for its appreciation but a knowledge of most elementary geometrical optics.

If a small fragment of muscle be teazed out in water, salt solution, or almost any other fluid, and examined in the ordinary way, with a power of 300 diameters or more, the important fact may be made out (which is the basis of all my future observations), that the borders of the fibres are not smooth, but undulate, presenting wavy margins. (Fig. 1.)

In the fresh unstained preparation there is a halo around the edge of the fibre which masks the crenulated border, yet by carefully adjusting the mirror so as to obtain oblique light, or by searching for a fibre partly in the shade of another, this may always be made out; in the case of insects' muscle, this is, however, always easy to demonstrate, for the fibres are much coarser, indeed, the appearance has been often figured in the works even of recent histologists. If the preparation be stained by any of the ordinary dyes, perhaps most readily by picro-carmine, the border is in all cases very distinct, and the regularly sinuous margin is unmistakeable. Now, what is the significance of the wavy outline? It is, as will readily be understood, that the fibre is ampullated, the wavy outline being but the optical expression of such a figure. A muscular fibre is then not a smooth cylinder, but is like the turned leg of a chair, or like the transversely ribbed neck of a common water-bottle in shape. If the fibre be broken up into fibrillæ, which is very easy, after maceration in alcohol, these are seen to have just the same characters, indeed, a small bundle of fibrils is most convenient for study. It may be well to remark, that the ultimate fibrillæ often show but little cross marking, and appear almost smooth; that is, however, only due to their small size; a good lens will bring out both points.

The above-described appearances may be observed in all the varieties of muscle that I have as yet examined, *e.g.*, those obtained from man, the dog, cat, rabbit, guinea-pig, mouse, frog, mussel, crab, bee, wasp, Dytiscus, Hydrophilus, common house fly, &c., &c.

The transverse stripings of the fibre are related to and correspond with the inequalities of the surface. (Fig. 1.) The little elevations at the borders correspond, of course, to the little ridges which run round the fibre, while the dips at the borders are the optical expressions of little valleys running between them. In the ordinary position, the dark stripe marks the position of the ridge, and the light stripe lies in the little valleys, as will be seen on reference to fig. 1, Plate 5.

Then, again, Dobie's line (Krause's membrane), which is a faint dark band in the very centre of the bright stripe, runs along the

bottom of the valleys (D in the diagram), and Hensen's stripe in the centre of the dark band, lies on the exact summit of the ridges. (H, fig. 1.)

This position of the stripes in a normal muscular fibre, is the invariable rule, and the idea at once suggested itself, may not the shape of the fibre itself cause the cross stripings?

Any student of natural philosophy would at once affirm that a structureless fibre of such a shape must be cross striped, and a glance at the ribbed neck of the water-bottle on the table will elicit the same answer from any one.

The question we must now determine is, are the appearances seen in the fibre just the same in all their details, as would be produced by a piece of glass, or any other homogeneous transparent substance of the same shape?

Before, however, entering into theoretical grounds, it may be as well to give a full description of what is actually to be seen, for this has yet not been stated.

With a structure of complicated figure, such as the one we are considering, it is obvious that there is no one focus in which it may be described. There is one pretty definite focus for a single speck or thin film, but even when examining a simple cylinder, it is evident that when the borders of it are clear and distinct, the upper surface is slightly out of focus. We shall see, that in the case of the muscle, although there is one position of the lens when the parts are very distinctly seen, and in which they have mostly been described, yet that on slightly altering the focus, the appearance is changed. These changes we must carefully study.

For this purpose we may select the large muscles of the thigh of a rabbit; stretch them ever so little upon a piece of wood, and place them for some days in 50 per cent. alcohol. A high power is required for their examination; I have been in the habit of using a $\frac{1}{24}$-inch of Gundlach, a very perfect lens; a $\frac{1}{10}$-inch will, however, do. A small bundle of fibrils should be selected in preference to a whole fibre for examination.

On focussing it becomes at once apparent that on varying the adjustment ever so little, you may bring into focus the tops of the ridges or the bottoms of the valleys which lie between them. Now this slight alteration is sufficient entirely to change the optical appearances.

First raise the lens until the fibre be out of focus and is only to be seen as a dim streak running across the field, then bring it down until its form and the cross markings are distinctly to be seen (the border is now not quite distinct on a level with the horizontal axis of the fibre). In this position alternating light and dark bands are made out, but no vestiges of Hensen's stripes or Dobie's lines. (Fig. 3, *a*.) The

dark band corresponds with the valley and the light one to the ridge, or crest. This was the focus in which Bowman described his preparations as far as I can gather from the paper. If the lens be now lowered ever so little, the stripes are reversed, a most curious point, which was noticed by Bowman, but afterwards lost sight of. The dark band now corresponds with the ridge, and the bright band with the valley. (Fig. 3, *c.*) This is the focussing in which it is usually described, and in this position Dobie's line and Hensen's stripe are to be seen as a rule in uncontracted fibres.

Between these two positions of the lens there is generally a well-marked intermediate one, which is depicted in fig. 3, *b*. The crests and valleys are both bright and equally so, although the slightest movement of the fine adjuster will make either one or the other the darker; on the slopes, as it were, there are, however, narrow shaded bands, which are shown in fig. 3, *b*. The fibre is now quite clear and distinct, and the longitudinal fibrillation is now best made out—if it can be seen at all—and yet there is no sign of either Hensen's or Dobie's stripes. These being the observed appearances (and they may be verified without very much trouble), I shall calculate theoretically the appearances which a homogeneous fibre of such a shape should present when examined by transmitted light, so as to see whether our observed effects tally with what may be theoretically calculated.

Parallel rays of light pass upwards through the fibre, and in their course are altered in direction (see fig. 4). The substance of the fibre being of higher refrangibility than the fluid in which it is mounted, the thicker parts which correspond to the ridges will act like converging lenses, causing the rays of light to come to a focus (A A′ A″), diverging again. The thinner parts (the valleys) will, on the other hand, act as diverging lenses, causing the rays to spread out, as may be seen on reference to the diagram. Now it is evident that when the objective is arranged to focus those rays which have passed through the fibre and converge over the ridges, at that same position the rays above the valleys will be diverging (see fig. 4). This will produce a difference in the appearance, for the converging rays will give a bright band, while the position of those rays which diverge will appear darker. Alter the focus by screwing the lens up or down, and, provided the fibre can still be seen, this state of matters will be reversed; for after converging, the rays above the position of the ridges will now be diverging, while at the same time those over the valleys will be converging and will appear bright.

The condition seen in fig. 3, *b*, which is intermediate between the low and high focussed picture of the fibre, would be obtained by shifting the lens half-way between these two positions. Hensen's stripe is no doubt due to rays passing through the centre of the ridges suffering little refraction in their course, and thus causing a brightness.

Dobie's line might, of course, be the reverse of this, no rays at this point coming to the eye of the observer; but we shall speak of this more hereafter, when we shall show that there is some reason for suspecting at this point a distinct structure.

Although it is indispensable to account theoretically for these appearances, yet to most persons a simple demonstration will carry more conviction than any proof deduced from the laws of optics, however well they be understood. Instead of showing "what should be," we will study "what is."

For this purpose we will imitate as nearly as possible the figure of a muscular fibre on a small scale, and it shall be made out of a substance of uniform consistence throughout. What appearances will it present on microscopic examination? I have proceeded in the following manner:—A glass rod is heated in a spirit-lamp and plunged into a bottle of Canada balsam; it is then withdrawn, and a little drop of the balsam is allowed to fall on a glass slide, or a thread of it may be laid out on the surface of the glass. Before the drop or thread has solidified it is indented with the milled head of a fine screw, and examined with a power of from twenty to fifty diameters, when cross shadings are to be observed. These are seen, moreover, to correspond with the surface impressions, and not only so, but they are reversed on altering the focus. Hensen's stripe is generally very well seen. The most beautiful and convincing object to study in this connexion is a scale of the Lepisma. These are sold as test objects with many microscopes. They are oval in shape, transparent, and singly refractile throughout, and beautifully ribbed in their length, these ribbings or groovings being indeed so fine that a power of at least 500 diameters will be required to make out those points to be here described. You would think on looking at one of these scales that a piece of muscle was flattened out before you on the field: no rough balsam model, but a perfect illustration taken from the back of a tiny insect.

The appearances it is needless to describe, for they are, almost to the minutest detail, those of a muscular fibre. The bright and dark stripe interchanging with every alteration of focus, Hensen's stripe, and Dobie's line (Krause's membrane) are all to be seen. In the case of the Lepisma scale the line of Dobie is in the centre of a bright band, which is broader than the dark band with Hensen's stripe. This is, of course, the other way in the case of the muscular fibre.

We see, therefore, that a muscular fibre presents just those appearances which a transparent body of uniform texture and of similar shape would possess. However conclusive these proofs may have been, it is well to collect all evidence possible to show that these markings are nothing more than optical effects, to which end a very searching experiment was suggested to me by Professor Tait. It is evident that if these cross bands are seen when parallel, or nearly

parallel, rays of light are passing through the fibre, by using converging or diverging rays the appearance will be altered, and it will be possible by careful adjustment of a lens to cause a total reversal of the striping. If a fibre be carefully focussed and a strong biconcave diverging lens be placed between the stage of the microscope and the mirror, and carefully moved about with the fingers, it will be possible entirely to alter the fibre, causing a total reversal of the cross bands. On withdrawing the lens, of course the fibre resumes its normal appearance. I may mention that several lenses were tried before one was found which would in at all a satisfactory manner show this phenomenon; when successful the experiment is very striking.

In opposition to my view is the one generally accepted, namely, that the cross stripings are produced by differences along the fibre of chemical composition, and refrangibility.

Now, suppose that there were along the fibre two alternating structures, A and B. Let A represent the bright stripe and B the dark stripe. If A has a higher or lower refractive index than B, it is evident that although they were immersed in any number of fluids of refrangibility varying from the lowest to the highest, yet A would always be distinguishable from B, and the striping would always be apparent. Then, again, by placing the fibres in fluids of indices near to that either of A or B, the more striking would be the contrast. If, however, the fibre were homogeneous throughout, the striping being nearly due to the form, then if the fluid and the fibre have the same refractive index all striping will disappear. On Professor Tait's suggestion, I tried a series of fluids formed by mixing, in various proportions, alcohol, whose refractive index is low, with oil of cassia, which is high. In this way I have prepared specimens showing almost no cross striæ, the fibre appearing uniform until after most careful examination.

Dr. Klein has since shown me some muscular fibres of an insect. They were quite smooth and cylindrical, and were unstriated. In these specimens there were, on very close examination, cross lines separated by comparatively wide intervals. It is possible that they represented Dobie's lines.*

But it may well be asked, What about the action of staining agents, such as logwood, which is stated to tint the dark stripe and Dobie's line? Does this not show a difference of structure along the fibres?

Once having the clue it will be understood that just as the unstained

* More recently my friends Messrs. Geddes and Beddard have demonstrated a very curious condition in the muscular fibres of the Echinus, which my views entirely explain. They noticed that in the same fibre some parts were cross striped, while in parts no striation was to be seen. Hearing of my explanation of the markings, they re-examined their specimens (which I have also seen), and found that when the striæ were visible there, and only there, the fibre was ampullated. (See fig. 5.)

fibre will modify and change the direction of rays passing through it, so will also a stained fibre produce what are apparently modifications of the staining effect. It is generally stated that the dark band and Dobie's line are stained by logwood and carmine, while the bright bands remain unaffected; also that Hensen's stripe in the centre of the dark stripe is stained only to a slight degree: whence it follows that if staining action is to be the criterion, this stripe differs in structure from the dark stripe.

We, however, affirm that the whole fibre is stained, and equally stained throughout. The bright band is undoubtedly stained, although it appears not of the deep blue of the dark stripe when coloured by logwood; and this conclusion is drawn not only from an examination of my own specimens, but also from some of great beauty shown to me by Dr. Klein. Why the bright band does not appear of so dark a blue is, that the apparent shading of the latter is added to the blue tint, producing a depth of colour. The most conclusive proof of this is, that one can often reverse the colouring on readjusting the focus, and that Hensen's stripe or the bright part of the dark stripe is only of a faint light-blue, like that of the bright stripe.

Picric acid stains muscle very readily, and colours it throughout. The fibre to the naked eye is yellow and uniformly so, but when examined by the microscope, alternating yellow and shaded yellow bands are to be observed, which reverse their position on changing the focus. With a high focus—when the crests are bright in the unstained preparation—they are of a bright yellow, while the valleys are of a deeper yellow tint.

To show the effects which a fibre of this shape can produce when transmitting monochromatic light, nothing can be more conclusive than the following experiment. A slip of coloured blue glass is held obliquely between the reflector and the stage of the microscope, so that blue rays pass through the fibre. It does not appear of a uniform tint, but beautiful blue stripes are seen corresponding with the crests and valleys, and varying with alterations of focus. If a piece of red glass be substituted for the blue slip, red cross stripes are seen in corresponding places. For this experiment the fresh fibres of insects' muscle should be examined, for, with fine mammalian muscle, the light is not so good, owing to the higher power required. This experiment has been introduced here with the description of stained muscle, not that it can be strictly compared with an ordinary staining process, but simply to show what an influence the fibre's shape must have upon the tinting, supposing, as we do, that this is in reality uniform.

An investigation such as this is beset with many difficulties and fallacies, and I may mention one which befel me in this stage of my work.

I had stained a few muscular fibres of a rabbit with picro-carmine,

and on examination, what was my surprise to find that in some of them the light stripes (valleys) were most brilliantly stained with carmine. I was long puzzled at this, when it was at last discovered that the picro-carmine had dried somewhat on the preparation, and the carmine had mechanically precipitated along the valleys, filling them up. At the end of one or two fibres this precipitation had partially peeled off, showing undoubtedly the true nature of the phenomenon.

I have in my possession very beautiful alcoholic preparations stained with logwood. At first sight, from a study of many of the fibres, one would be led to believe that the bright stripe is wholly unstained, while the dark stripes are of a beautiful violet.

A careful examination, however, reveals the fact that such fibres are broken up transversely, looking like piles of coins, a very common occurrence, especially in preparations that have been long mounted. The coins, lying close to one another, with narrow chinks between, of course revealed transverse unstained tracts, which could well be mistaken for the bright stripe.

More interest and discussion has hitherto accrued to the action of muscle on polarised light, than to the effects of staining reagents. We have seen that much difference of opinion exists: Brücke has maintained that not only is the dark stripe (ridge), as all are agreed, doubly refracting, but that the whole of the light stripe is isotropous. I myself was led to modify this, discovering that on careful focussing with a fibre not at all sheared in its length, the central part of the light stripe was undoubtedly anisotropous. This I have afterwards seen figured, as before mentioned, in Hermann's "Physiology," and have introduced the diagram into fig. 2. It is a point of some practical difficulty to mark exactly the positions of the cross bands while turning the analyser, and thus changing the character of the field. This difficulty has been overcome completely by a suggestion of Professor Tait's, who has helped me much in this part of the work. Very fine emery powder should be sprinkled over the preparation before covering it; for then, on examination, numberless little black specks will be seen in the field. A cross band of a fibre is selected for examination which is exactly opposite one of these little specks, then when you rotate you can definitely affirm, having the little black speck for your guide, what change has occurred.

Rabbits' muscles are very satisfactory objects for examination, as they do not cleave across at all readily. The adductor muscles of the leg should be excised, slightly stretched on a piece of wood, and placed in 50 per cent. alcohol until they split readily into fibrils. They may then be mounted in any ordinary fluid, a pinch of emery powder having been sprinkled over the preparation before covering.

It is necessary to use a power of 800 or 1,000 diameters in the in-

vestigation of mammalian muscle, while in the case of the insect one of 300 diameters is quite enough.

In the living and dead muscular fibre the whole of its substance is doubly refracting. The observations of some modern observers entirely agree with my own, in that, with crossed Nicols, the crests (dark bands) and the centres of the valleys (bright stripes) appear bright and therefore refract light doubly, and that there are two dark bands on the slopes between them. (See fig. 2.) It does not follow, however, that these two dark bands represent tracts of isotropous substance. This is the point at issue. The dark lines between the valleys and ridges which appear when the Nicols are crossed have been interpreted as marking the positions of cross bands of singly refracting substance, but this is a fault of reasoning. If the fibre were smooth and cylindrical it would then follow, but the fibre is not, as we have already insisted. These bands lie just on the sloped parts of the fibre, those sections in fact which are oblique to the passing rays; and the explanation is now quite easy, for the extraordinary ray passing through the fibre is naturally deflected at these parts, and does not reach the eye of the observer. Hence the body appears not to transmit them at all at these parts.

It is not difficult to explain the discrepancies between Brücke's description of the bright stripe and my own.

It is essential to be very scrupulous in the selection of a fibre for examination. It must not be at all twisted, or sheared in the slightest degree, for then the cross stripes are not at right angles to the long axis, and as their width is several times their thickness (in the length) overlapping will to some extent occur. This will certainly lead to very confusing results, and the bright centre of the bright stripe (valley) may well be overlooked. Moreover, the fibre should be slightly stretched and as small as possible.

It has previously been mentioned that in many preparations the fibres split up transversely in a most regular manner, and unless the cover-glass be pressed upon, the little disks remain in position with narrow chinks between them. These chinks will be filled with the isotropous fluid used for mounting, which will lead to very anomalous appearances, and which may perhaps help to account for some of Brücke's statements. These fallacies may be avoided by a study of the fresh fibres of insects' muscle. Dytiscus and Hydrophilus muscle has received a large share of the attention of histologists, but that from the wasp or blue-bottle fly is quite as good. A leg should be pulled from the trunk of a blue-bottle fly and this again forcibly separated at the middle joint. A piece of muscle will project from one of the segments, which may be cut off and examined in a drop of fluid expressed from the thorax of the fly. The polariscopic effects may then be made clearly out in the still contracting fibres. I have

tested all these points by a careful examination of insects' fibres with thin plates of selenite and mica. This method is not so satisfactory, nor do the differences of colour seen give such trustworthy evidence as may be obtained by the crossed Nicols alone.

The Fibre during Contraction.—Living insects' muscle may be examined and the changes observed when the waves of contraction pass along the fibre, or perhaps better still, they may be fixed with osmic acid. The muscles from the leg of an insect are rapidly separated out on a slide, and a drop of weak osmic acid added which kills the fibres instantaneously, fixing them in the position that they happen to be in. On examination one generally finds fibres which in part of their course are contracted, and in other parts relaxed, when the differences in appearance may readily be studied. It may here be observed that the fibres bulge at the contracted part, so that if the surfaces be examined the focus of the microscope must be accommodated.

The cross stripes are nearer one to another and correspond, as before, with the ridges, and valleys seen at the margin, which are much more prominent and bolder in outline.

In the Contracted Fibre the Striping is practically the same as in the Stretched Condition.—The contracted fibre exhibits just the same reversing of stripes on alteration of focus, and Dobie's line and Hensen's stripe can both be seen in the same positions as in the uncontracted muscle, provided the fibre is suitably placed for examination and not sheared in its length. We must entirely deny the common statement, first introduced, we believe, by Merkel and Engelmann, that in the contracted state the bright band becomes the darker. If good specimens of insects' muscle be examined, which have been treated with osmic acid, and if the fibre be not sheared, the valley is always bright in the ordinary or deeper focus. I have verified this point in very many cases. Passing along a fibre from the relaxed end to a part where the contraction is fullest, the appearances vary in degree, but not in kind. The main features are in both cases the same, but the stripes are now narrower, and often it is not so easy to see Dobie's and Hensen's stripes. This follows from the statement of Engelmann, viz., that "the bright stripes become darker than the dim;" for he himself notices that at one point, or phase, in the contraction, no striping is to be made out. We agree with Ranvier that this is not true, indeed it would be impossible for a muscular fibre with its configuration not to be marked across its length.

This subject will call up to the mind of every working histologist, appearances which he must have met with in other fields of research. Many tissues naturally, or after clumsy manipulation, present ampullations which always co-exist with cross striæ. The fibres of the crystalline lens are wavy in outline, and when many of them are

bound together and seen on edge with the wavy outline towards the eye of the observer, cross bands are seen which in chance preparations (especially those of the frog's lens) simulate muscle in a wonderful manner. Ordinary non-striped muscle which may be so well seen in the frog's bladder is often faintly ampullated especially, perhaps, in chloride of gold preparations. Cross stripes may also here be seen. The fibres of Tomes, when a section of softened tooth is teazed, are pulled out of the dentinal tubules, and, being of a soft and somewhat elastic nature, on breaking they become often very beautifully ampullated, and it would be impossible to distinguish them from muscular fibrillæ. In the class of practical histology, on more than one occasion, students have asked me the meaning of beautiful cross shadings seen on nerve fibres; a slight ampullation which fully accounted for it, was always found.

Many more of such instances could be recalled in the experience of every one; it is needless to enumerate further.

In the winter of 1879–80, while examining fibres of the muscles of a newly-born child, a very curious discovery was made. A nucleus belonging to the sarcolemma was seen beautifully striped. It was not in close apposition to the fibre, a very narrow chink intervening. On focussing with great care, it was seen that the cross bands upon it corresponded with those of the adjoining fibre, a dark one, however, for a light one, and *vice versâ*. (Fig. 6.) Now, the curious point was that the nucleus had evidently been impressed by the fibre, moulded upon it, as it were, and on being pulled apart had presented a perfect cast of the surface. One would hardly believe in sarcous elements here. Last summer (1880) my friend Mr. Priestley communicated to me a similar and independent observation of his own, as a contribution towards the maintenance of my views upon the formation of the stripes.

The position that we have reached is this: a muscular fibre presents such cross markings, varying with shifting the lens up or down, as a filament of homogeneous structure and similar shape. I have shown this experimentally, and have illustrated it by simple experiments, which it is in the power of anyone to test. This being the case, I have searched to find if there be reason to assert any want of uniformity along the fibre, using various methods of staining. This I have failed to do, and have shown that the views commonly held are to be explained simply by the shapes of the fibres. As to the action of muscle on polarised light, I saw reason to dissent from the views of Brücke, and subsequently found my own in accordance with those of other recent observers. I differ from them in the explanation I offer of the two dark bands seen with crossed Nicols, for here, again, the shape of the fibre explains their presence without looking for any special structure.

So far we are led to consider the fibre as made up of many ampullated fibrils, packed side by side, forming an ampullated fibre, these fibrils being uniform throughout, and joined together by some cementing material, the nature of which we will not surmise. The only point which would suggest a definite structure along the fibril is the attachment of the sarcolemma in insects' muscle to Dobie's lines. There is no doubt that this membrane dips down and seems prolonged into Dobie's lines in a most beautiful and regular manner. The significance of this is very obscure, and is quite beyond me. There are many possibilities. It may be, although there is no proof of it, that a membrane exists here continuous with the sarcolemma; it may be that there is nothing but some cementing substance more soluble in alcohol than the sarcous matter: it may be that there is a little minor crest at this point to which the sarcolemma is attached. This little crest I have certainly seen in some fibres, and it has already been figured by more than one writer, yet in other fibres, the outlines of which are wonderfully distinct, no trace of it is to be made out. The fibres can hardly be said to break across in the line of Dobie, all that can definitely be affirmed is that they cleave in the thinnest part, or the light stripe. The investigation of this point is one of great difficulty, owing to the haze around the broken points, and I can never make up my mind to any definite statement. This transverse cleavage is not of course a point of very much weight, as the fibre would naturally tend to split across in or near Dobie's lines, as here it is thinnest.

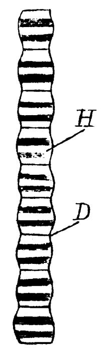
The striping of muscle can be easily explained, as shown before, which leads me to my final statement. A fibril is structureless throughout its entire length, except that, perhaps, there may be membranes, or lines of fission, or layers of cement at the positions of the lines of Dobie; this we leave an open question. In using the word "structureless," I must not be misunderstood; structureless membranes and tissues are fast losing their place in histology, and once simple protoplasm is now most complex. What I infer is that the stripes do not mark the positions of alternating layers of different structure, the presence of which are ordinarily maintained. The complicated "Muskelkästchen" of the Germans do not exist.

The muscular tissue of the heart presents many peculiarities which it is needless here to enumerate, for the cross striping alone concerns us. All those cross bands which have been described in ordinary voluntary muscle may here also be seen, and they are placed in the same relations with the turned surface of the fibre. The dark stripe corresponding to the crests, or ridges, the light bands to the depressions between them. (Fig. 7.) Dobie's lines may be made out with great ease, and as there is no sarcolemma here, they may be accounted for also purely from the shape of the fibres. I have often thought that Dobie's lines marked the positions of tiny ridges in the valleys, but this is a

point more difficult to decide perhaps than in the case of the skeletal muscles. Transverse cleavage takes place here also in the thinner part of the fibre, namely, in the bright stripe, but whether or not exactly in Dobie's line I have not yet definitely made out.

A curious appearance often presented by insects' muscle, and sometimes also by that of the mammalia, has been described and figured by Mr. Schäfer. A paper descriptive of these he communicated to the Royal Society of London (1873), which came out later on in the "Transactions" of this Society, and his observations are published also in the eighth edition of Quain's "Anatomy." These have been almost entirely overlooked by French and German physiologists, yet in many English laboratories his observations have been verified, and his conclusions taught.

They are well illustrated in a representation of the muscular fibre of a Dytiscus, which may be seen in Quain's "Anatomy." The dark stripes are traversed longitudinally by dark rods, which end at both extremities in little knobs. These knobs lie in the borderland between the bright and dark stripes. The only point which I would add to his figure is this, that the knobs are joined across the clear stripes or valleys by lines, just as they are so joined across the dark stripes, although the lens must be depressed ever so little to make this out. These lines are, in fact, nothing more or less than the longitudinal striæ described many years ago as lying between the fibrillæ of which the fibre is composed, these little knobs lying in their course. This can, perhaps, most conclusively be made out in the following way. Allow a piece of insect's muscle to remain in a drop of water for some hours (which will vary with the temperature) until it has partially putrefied. Then cover and examine, when many of the fibres will have separated towards their ends into fibrillæ. One can then distinctly trace the chinks between the separated fibrillæ as being continuous with the striæ, on which the knobs are still seen, in the centre of the fibre. I think that the following is a feasible explanation of these knob-like enlargements of the cementing substance seen as longitudinal striæ. These knobs occur, as will be beautifully seen on referring to the woodcut in Quain, on the slopes between the valleys and the ridges. The cementing substance dips down here with the fibre itself, and if there be the slightest lateral obliquity it will appear larger. The cementing matter is seen on edge, and differing as it does from the muscle-substance in refrangibility, a distortion occurs, giving rise not to a dark line as on the surface, but to a dark knob. This is, in fact, but an optical delusion, for the striæ are quite uniform, and were the fibre cylindrical would appear so. This may be proved by the fact that very often if the rays of light from the reflector are oblique, but one set of dots appears, which shift over to the other side on twisting the mirror. By shifting the preparation



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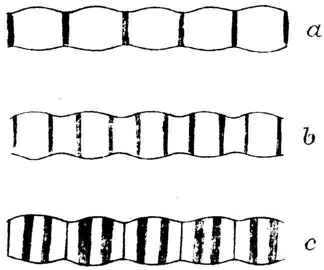

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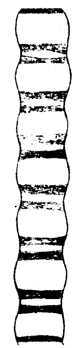

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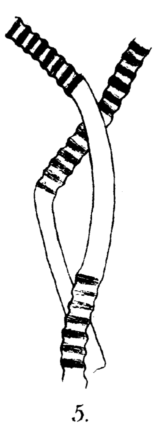

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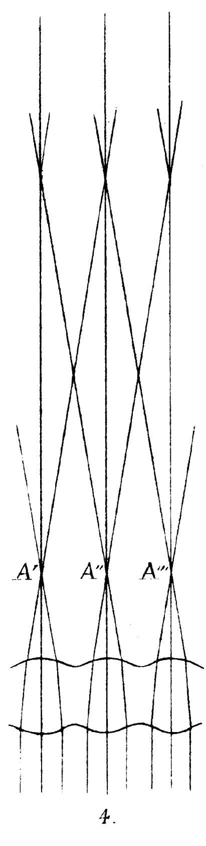

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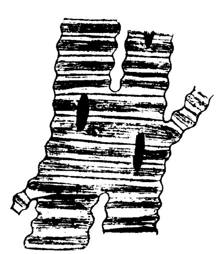

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about, or by twisting the tube of the microscope obliquely, the dots disappear from one part of the fibre to appear in another, showing that it is but an optical effect, and that no structure here exists.

Before concluding I must gratefully acknowledge much help and sympathy which I have received in this investigation.

To Professor Tait I have gone when in any difficulty, for an observer in a case such as this must have the aid of an experienced physicist, otherwise grievous error is but courted. To him, as has been seen in the text, I owe many suggestions, and he has kindly entirely looked over my paper. Dr. Klein has shown me great kindness in carefully examining my preparations from the histological point of view, and as has before been mentioned, in showing me preparations to corroborate my views. My thanks are also due to my friend Mr. John Priestley, for many hints, especially concerning the literature of the subject.

DESCRIPTION OF PLATE 5.

Figure 1. This represents a muscular fibre viewed with a very high power. The borders are wavy and the cross stripes correspond with these inequalities. (D) marks the positions of Dobie's dark stripes placed in the centres of the depression seen at the border. (H) represents Hensen's stripes, or Dobie's light stripes, placed on the summit of the ridges, in the centre of the dark band.

Figure 2 shows the appearance of the fibre with crossed Nicols. The shaded parts are seen on the slopes between the ridges and depressions. They are explained fully in the text.

Figure 3. A fibre is represented as seen with three positions of the lens. In (*a*) the lens is elevated and the depressions appear dark. In (*c*) the lens is fully depressed when the stripes are reversed, the depressions being now light with Dobie's dark stripe in the centre of them, and the crests dark but with Dobie's light stripe in the midst. In (*b*) an intermediate stage is seen.

Figure 4. This shows the passage of rays of light through the fibre. The convex parts converge the rays to focus A', A'', A''', after which they diverge. The lens shifted up or down (vertically) over the ridges or depressions, will focus on the retina alternately converging and diverging rays.

Figure 5. Muscular fibres of Echinus, described by Messrs. Geddes and Beddard.

Figure 6 represents a nucleus seen by me, which impressed on the muscle, is moulded to the same shape and appears to be cross striped.

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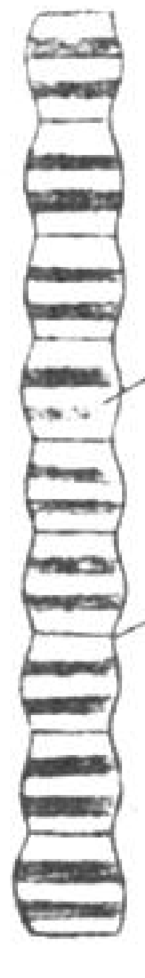

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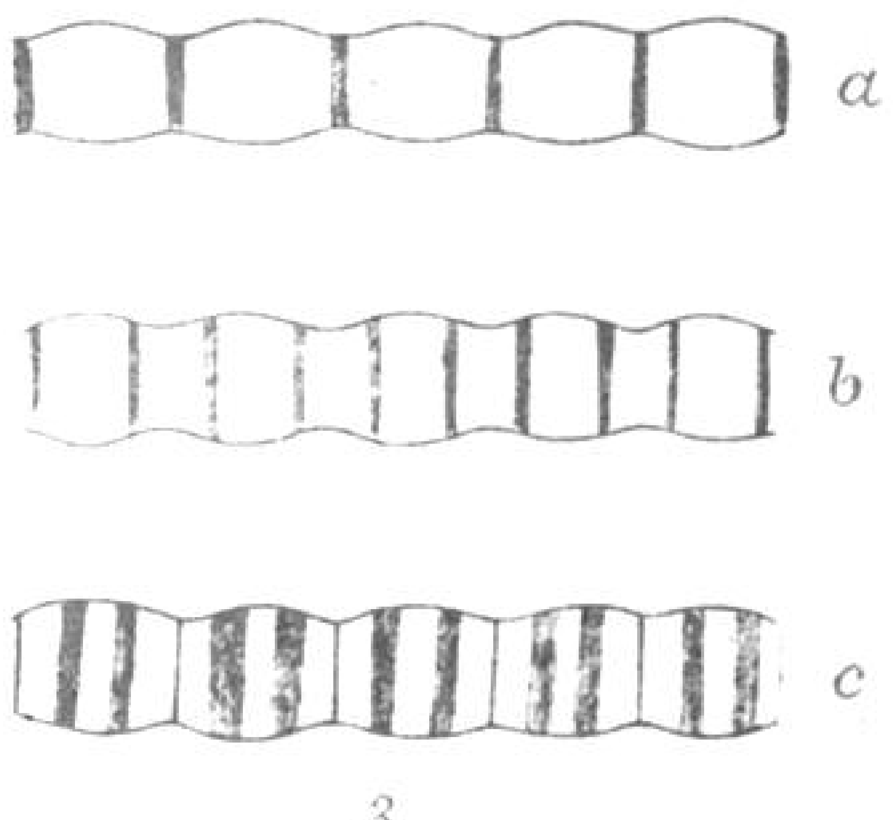

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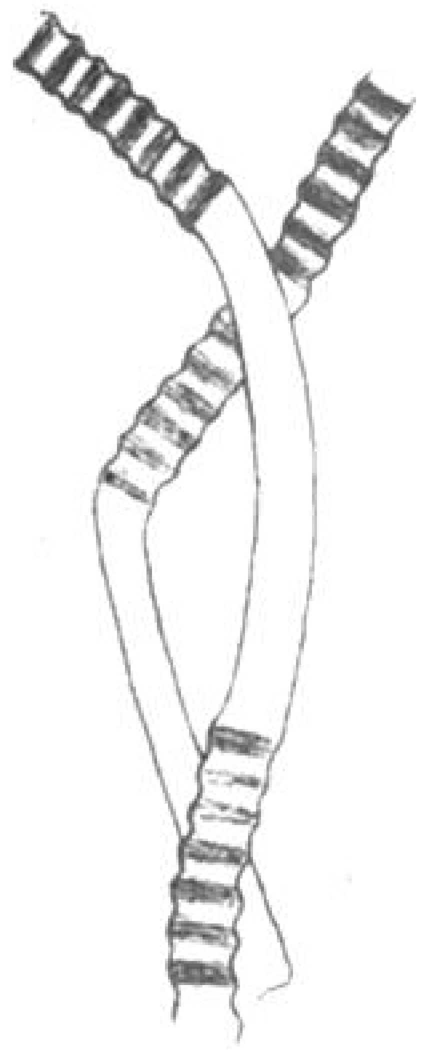

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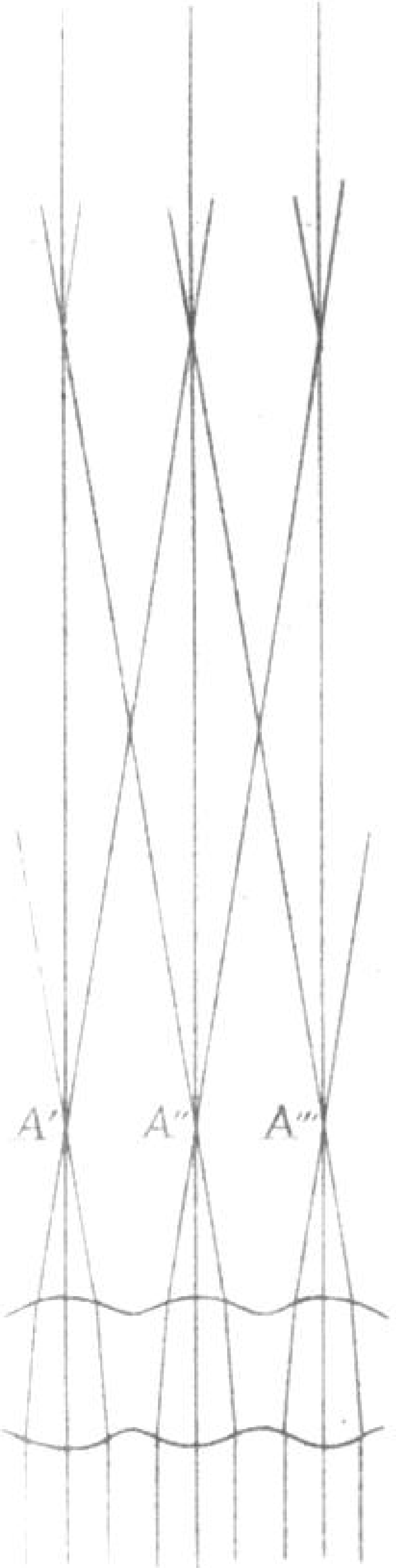

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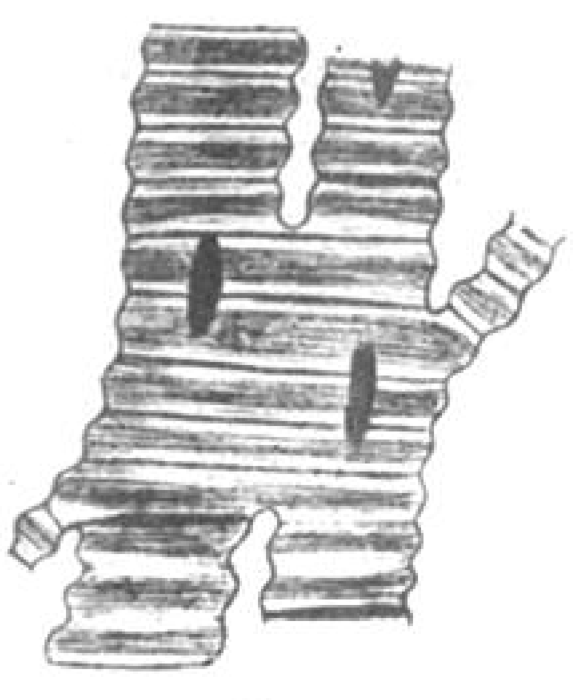

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