

XXXIII.—*On the Minute Structure of Involuntary Muscular Fibre.* By JOSEPH LISTER, Esq., F.R.C.S. Eng. and Edin., Assistant-Surgeon to the Royal Infirmary, Edinburgh. Communicated by Dr CHRISTISON.

(Read 1st December 1856.)

It has been long known that contractile tissue presents itself in the human body in two forms, one composed of fibres of considerable magnitude, and therefore readily visible under a low magnifying power, and marked very characteristically with transverse lines at short intervals, the other consisting of fibres much more minute, of exceedingly soft and delicate aspect, and destitute of transverse striæ. The former variety constitutes the muscles of the limbs, and of all parts whose movements are under the dominion of the will; while the latter forms the contractile element of organs, such as the intestines, which are placed beyond the control of volition. There are, however, some exceptions to this general rule, the principal of which is the heart, whose fibres are a variety of the striped kind.

Till within a recent period the fibres of unstriped or involuntary muscle were believed to be somewhat flattened bands of uniform width and indefinite length, marked here and there with roundish or elongated nuclei; but in the year 1847, Professor KÖLLIKER of Würzburg announced that the tissue was resolvable into simpler elements, which he regarded as elongated cells, each of somewhat flattened form, with more or less tapering extremities, and presenting at its central part one of the nuclei above mentioned. These “contractile” or “muscular fibre-cells,” as he termed them, were placed in parallel juxtaposition in the tissue, adhering to each other, as he supposed, by means of some viscid connecting substance. In the following year the same distinguished anatomist gave a fuller account of his discovery in the 1st volume of the *Zeitschrift für Wissenschaftliche Zoologie*, and described in a most elaborate manner the appearances which the tissue presented in all parts of the body where unstriped muscle had been previously known to occur, and also in situations, such as the iris and the skin, where its existence had before been only matter of conjecture, but where the characteristic form of the fibre-cells, and of their “rod-shaped” nuclei had enabled him to recognise it with precision. Confirmations of this view of the structure of involuntary muscular fibre were afterwards received from various quarters, one of the most important being the observation made in 1849 by REICHERT, a German histologist, that dilute nitric or muriatic acid loosens the cohesion of the fibre-cells, and enables them to be isolated with much greater facility. In 1852 I wrote a paper “On the Contractile Tissue of the Iris,” published in the *Micro-*

scopical Journal, in which I gave an account of the involuntary muscular fibre contained in that organ in man and some of the lower animals, stating that the appearances I had met with corresponded exactly with KÖLLIKER's descriptions, and illustrating my remarks with careful sketches of several fibre-cells from the human iris, isolated by tearing a portion of the sphincter pupillæ with needles in a drop of water. In 1853, another paper by myself appeared in the same *Journal*, "On the Contractile Tissue of the Skin," confirming KÖLLIKER's recent discovery of the "*arrectores pili*," and describing the distribution of those little bundles of unstriped muscle in the scalp. These and other investigations into the involuntary muscular tissue convinced me of the correctness of KÖLLIKER's observations, and led me to regard his discovery as one of the most beautiful ever made in anatomy; and this is now, I believe, the general opinion of histologists.

Still, however, there are those who are not yet satisfied upon this subject. In MÜLLER's Archives for 1854, is a paper by Dr J. F. MAZONN of Kiew, in which the author expresses his belief that the muscular fibre-cells of KÖLLIKER are created by the tearing of the tissue in preparing it, and denies the existence of nuclei in unstriped muscle altogether; but he gives so very obscure an account of his own ideas respecting the tissue, that his objections seem to me to carry very little weight, more especially as the appearances which he describes require, according to his own account, several days' maceration of the muscle in acid for their development. In June of the present year (1856), Professor ELLIS of University College, London, communicated to the Royal Society of London a paper entitled "Researches into the Nature of Involuntary Muscular Fibre." In the abstract given in the "Proceedings" of the Society, recently issued, we are informed that, "having been unable to confirm the statements of Professor KÖLLIKER respecting the cell-structure of the involuntary muscular fibre, the author was induced to undertake a series of researches into the nature of that tissue, by which he has been led to entertain views as to its structure in vertebrate animals, but more especially in man, which are at variance with those now generally received." In the "summary of the conclusions which the author has arrived at," we find the following: "In both kinds of muscles, voluntary and involuntary, the fibres are long, slender, rounded cords of uniform width" "In neither voluntary nor involuntary muscle is the fibre of the nature of a cell, but in both is composed of minute threads or fibrils. Its surface-appearance, in both kinds of muscle, allows of the supposition that in both it is constructed in a similar way, viz., of small particles or "sarcous elements," and that a difference in the arrangement of these elements gives a *dotted* appearance to the involuntary, and a transverse striation to the voluntary fibres." "On the addition of acetic acid, fusiform or rod-shaped corpuscles make their appearance in all muscular tissue; these bodies, which appear to belong to the sheath of the fibre, approach nearest in their characters to the corpuscles belonging to the yellow or elastic fibres which pervade va-

rious other tissues; and from the apparent identity in nature of these corpuscles in the different textures in which they are found, and especially in voluntary, as compared with involuntary muscle, it is scarcely conceivable that in the latter case exclusively they should be the nuclei of oblong cells constituting the proper muscular tissue."

Mr ELLIS, then, agrees with MAZONN in believing that the tapering fibre-cells of KÖLLIKER owe their shape to tearing of the tissue; and he regards the nuclei as mere accidental accompaniments of the proper muscular structure, probably belonging to the sheath of the fibres, which, according to him, are of rounded form and uniform width.

The distinguished position of Mr ELLIS as an anatomist makes it very desirable that his opinion on this important subject should be either confirmed or refuted, and the object of the present paper is to communicate some facts which have recently come under my observation, and which, I hope, may prove to others as unequivocally as they have done to myself, the truth of KÖLLIKER's view of this question.

In September last, being engaged in an inquiry into the process of inflammation in the web of the frog's foot, I was desirous of ascertaining more precisely the structure of the minute vessels, with a view to settling a disputed point regarding their contractility.

Having divided the integument along the dorsal aspect of two contiguous toes, I found that the included flap could be readily raised, so as to separate the layers of skin of which the web consists, the principal vessels remaining attached to the plantar layer. Having raised with a needle as many of the vascular branches as possible, I found, on applying the microscope, that they included arteries of extreme minuteness, some of them, indeed, of smaller calibre than average capillaries. A high magnifying power showed that these smallest arteries consisted of an external layer of longitudinally arranged cellular fibres in variable quantity, an internal exceedingly delicate membrane, and an intermediate circular coat, which generally constituted the chief mass of the vessel, but which proved to consist of neither more nor less than a single layer of muscular fibre-cells, each wrapped in a spiral manner round the internal membrane, and of sufficient length to encircle it from about one-and-a-half to two-and-a-half times. Fig. 18. (Plate XV.) represents one of these vessels as seen under a rather low power, and shows the general spiral arrangement of the fibres of the middle coat. Fig. 19. is a camera lucida sketch of the same artery highly magnified, in which I have for the most part traced the outline of the fibres on the nearer side of the vessel only, but one fibre-cell is shown in its entire length wrapped round nearly two-and-a-half times in a loose spiral. In some other vessels the muscular elements were arranged in closer spirals, as in figs. 20 and 21. They are seen to have more or less pointed extremities, and are provided with an oval nucleus at

their broadest part, discernible distinctly, though somewhat dimly, without the application of acetic acid. The tubular form of the vessels enables the observer, by proper adjustment of the focus, to see the fibre-cells in section; they are then observed to be substantial bodies, often as thick as they are broad, though the latter dimension generally exceeds the former. Here and there a nucleus is so placed in the artery as to appear in section with the fibre-cell, as shown in figs. 20, 22, and 23. The section of the nucleus is in such cases invariably found surrounded by that of the substance of the fibre-cell, though occasionally placed eccentrically in it. From the circular form of its section the nucleus appears to be cylindrical. These fibre-cells are from $\frac{1}{200}$ inch to $\frac{1}{100}$ inch in length, from $\frac{1}{2600}$ inch to $\frac{1}{2000}$ inch in breadth, and about $\frac{1}{2500}$ inch in thickness, measurements on the whole rather greater than those given by KÖLLIKER for the human intestine, the chief difference being that in the frog's arteries they are somewhat broader and thicker.

Now, the middle coat of the small arteries is universally admitted to be composed chiefly of involuntary muscular fibre; but in the vessels just described it consists of nothing whatever else than elongated, tapering bodies, corresponding in dimensions with KÖLLIKER's fibre-cells, and each provided with a single cylindrical nucleus embedded in its substance. Considering, then, that no tearing of the tissue had been practised in the preparation of the objects, but that the parts were seen undisturbed in their natural relations, it appeared to me that the simple observation above related settled the point at issue conclusively.

It was, however, suggested to me by an eminent physiologist, that the various forms in which contractile tissue occurs in the animal kingdom forbid our drawing any positive inference regarding the structure of human involuntary muscle from an observation made on the arteries of the frog. Being anxious to avoid all cavil, and understanding that Mr ELLIS's researches had been directed chiefly to the hollow viscera, I thought it best to examine the tissue in some such organ. For this purpose I obtained a portion of the small intestine of a freshly killed pig, selecting that animal on account of the close general resemblance between its tissues and those of man. The piece of gut happened to be tightly contracted, and on slitting it up longitudinally, the mucous membrane, which was thrown into loose folds, was very readily detached from the subjacent parts. I raised one of the thick, but pale and soft fasciculi of the circular coat, and teased it out with needles in a drop of water, reducing it without difficulty to extremely delicate fibrils. On examining the object with the microscope, I found that it was composed of involuntary muscular fibre, almost entirely unmingled with other tissue, reminding me precisely of what I had seen in the human sphincter pupillæ, except that the appearances were more distinct, especially as regards the nuclei, which were clearly apparent without the application of acetic acid. Several of the fibre-cells were isolated in the first specimen I ex-

amined, each one presenting tapering extremities about equidistant from a single elongated nucleus. The fibre-cells were of soft and delicate aspect, generally homogeneous or faintly granular, with sometimes a slight appearance of longitudinal striæ, such as is represented in fig. 4.

I had now seen enough to satisfy my own mind that the involuntary muscular fibre of the pig's intestine was similarly constituted with that of the human iris and the frog's artery: but before throwing up the investigation, I thought it right to examine carefully some short, substantial-looking bodies of high refractive power, which at first sight appeared, both from their form and the aspect of their constituent material, totally different in nature from the rest of the tissue. Several of these bodies are represented in figs. 10–15. Each is seen to be of somewhat oval shape, with more or less pointed extremities, and presents several strongly marked, thick, transverse ridges upon its surface; and each, without exception, possesses a roundish nucleus whose longer diameter lies across that of the containing mass. Yet between these bodies and the long and delicate homogeneous fibre-cells above described, every possible gradation could be traced. Figs. 8 and 9, are somewhat longer than those just indicated, and are also remarkable for their regularity. In figs. 5, 6, and 7, are represented fibre-cells of considerable length, marked here and there with highly refracting transverse bands, in the intervals of which they are of soft and delicate aspect. In several cells one half was short, with closely approximated rugæ, the other half long and homogeneous. Hence it was pretty clear that the appearances in question were due to contraction of the fibre-cells, and that the shortest of these bodies were examples of an extreme degree of that condition; their substantial aspect and considerable breadth being produced by the whole material of the long muscular elements being drawn together into so small a compass. The rounded appearance of the nuclei was accounted for by supposing either that they had themselves contracted, or that they had been pinched up by the contracting fibres, of which explanations the latter appears the more probable.

In order to place the matter if possible beyond doubt, I prepared two contiguous portions of the circular coat of a contracted piece of intestine in different ways; the one by simply cutting off a minute portion with sharp scissors, so as to avoid as much as possible any stretching of the tissue, the other by purposely drawing out a fasciculus to a very considerable length, and then teasing it with needles. In the former preparation, the fibre-cells appeared all of them more or less contracted, except in parts where the slight traction inseparable from any mode of preparation had stretched the pliant tissue, which in the fresh state appears to yield as readily to any extending force as does a relaxed muscle of a living limb. In the other object, where the tissue had been purposely stretched, most of the fibre-cells were extended, and possessed elongated nuclei. Here and there one

would be seen of excessive tenuity, scarcely broader at its thickest part than the nucleus, looking, under the highest magnifying power, like a delicate thread of spun glass. To how great a length the fibre-cells admit of being drawn out in this way without breaking I cannot tell. Fig. 1 represents a portion of such a fibre with the contained nucleus. Among these extended fibres, however, there lay, here and there, an extremely contracted one, the result, I have no doubt, of the irritation produced by the needles upon the yet living tissue. In order to guard against this source of fallacy, I kept a piece of contracted gut 48 hours, and then examined two contiguous parts of the circular coat in the way above described. The muscle was much less readily extended than in the fresh state, and I found that, where stretching of the tissue had been avoided as much as possible, it was composed entirely of fibre-cells marked with transverse ridges of varying thickness and proximity; a minute fibril having, under a rather low power, the general aspect represented in fig. 17. But I saw no distinct examples of the extreme degree of contraction so frequent in muscle from the same piece of intestine in the fresh state. This confirmed my suspicion that the latter had been induced by the irritation of the mode of preparation. On the other hand, a fully stretched fasciculus showed its fibres everywhere destitute of transverse rugæ, so that the point was now distinctly proved. KÖLLIKER, in his original article in the *Zeitschrift für Wissenschaftliche Zoologie*, figured some long fibre-cells with transverse lines upon them,—“knotty swellings,” as he termed them, which he supposed probably due to contraction, and he repeats this hypothesis in the part of his *Mikroskopische Anatomie*, published in 1852. The *proof* of the correctness of this idea is now, I believe, given for the first time.

The bearings of these observations on the main question respecting the structure of involuntary muscular fibre are obvious and important. In the first place, if the short, substantial bodies were mere contracted fragments of rounded fibres of uniform width, we should expect them to be as thick at their extremities as at the centre, instead of which they are always more or less tapering, and often present a very regular appearance of two cones applied to each other by their bases. Secondly, the uniform central position of the nuclei in the contracted fibres, proves clearly that the former are no accidental appendages of the latter, to which it seems difficult to refuse KÖLLIKER's appellation of *cells*.

The effect of acetic acid on the involuntary muscular tissue is to render the fibres indistinct, but the nuclei more apparent; and if this reagent be applied to a piece of contracted muscle, many of the nuclei are seen to be of more or less rounded form. The deviation of the nuclei from the “rod-shape” has hitherto been a puzzling appearance, but is now satisfactorily accounted for.

In examining a fasciculus that had been fully stretched, 48 hours after death, I met with several good specimens of isolated fibre-cells, two of which are represented in figs. 2 and 3. I would draw particular attention to the delicate, spirally-twisted extremities of the fibre-cell 3, such as no tearing of a continuous fibre could possibly have produced. Though these fibres are very long, yet we have no reason to believe that anything near the extreme degree of extension has been attained in them, and we cannot but contemplate with amazement the extent of contractility possessed by this tissue.

In fig. 16 is represented a portion of a fibre-cell curled up, which has been introduced for the sake of the clear manner in which it shows the position of the nucleus embedded in it. Just as in the case of the fibres wrapped round the arteries of the frog's foot, this cell might be seen in section by proper adjustment, and that section is observed to be oval; proving that the fibre is not round, but somewhat flattened. It happens that the nucleus appears at this point; its section is circular, and is surrounded on all sides by the substance of the cell.

The pig's intestine seems to be a peculiarly favourable situation for the investigation of unstriped muscle. Judging from KÖLLIKER's measurements, the fibres appear to be of much larger size there than in the same situation in the human body. The length of the fibre-cell 3 is $\frac{1}{37}$ inch. The fibre 2 is imperfect at one extremity; but, taking the double of the distance from its pointed end to the nucleus, its length is $\frac{1}{33}$ inch. These measurements are between three and four times greater than any which Professor KÖLLIKER has given for the human intestine, and considerably exceed the length of the "colossal fibre-cells" which he describes as occurring in the gravid uterus. The individual fibre-cells, with their nuclei and transverse markings, if they have any, are quite distinctly to be seen with one of SMITH and BECK'S $\frac{1}{16}$ object-glasses. But in order to examine their structure minutely, a higher power is required: that which I use is a first-rate $\frac{1}{12}$, made several years ago by Mr POWELL of London. All the figures in Plate XV., except 17 and 18, are from camera lucida sketches, reduced to the same scale. The principal measurements of the fibre-cells from the pig's intestine are as under:—

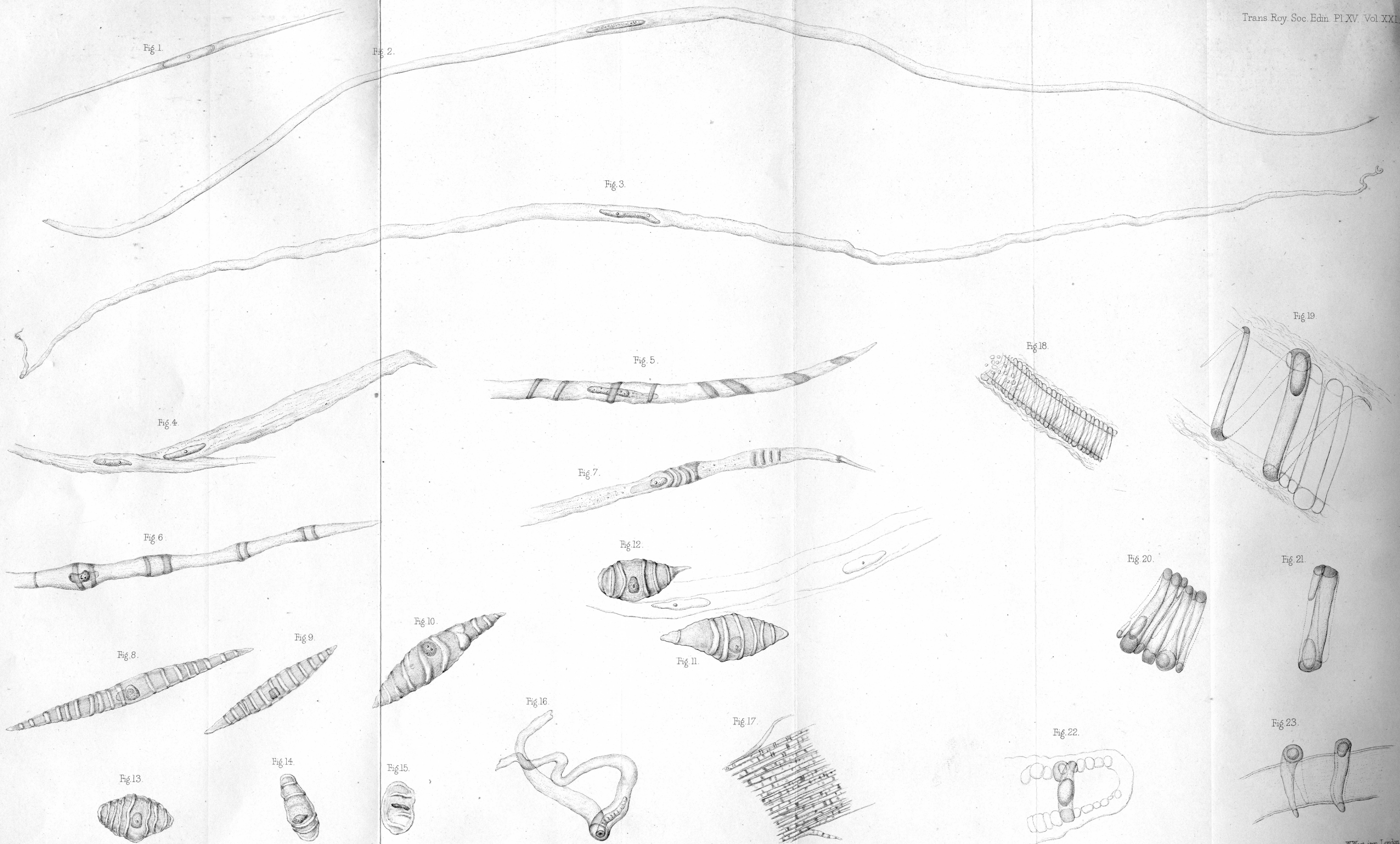
Length of fibre-cell, 3,	$\frac{1}{37}$ inch.
Breadth of ditto,	$\frac{1}{3300}$ "
Length of nucleus of ditto,	$\frac{1}{1000}$ "
Breadth of ditto,	$\frac{1}{8000}$ "
Breadth of fibre-cell, 16,	$\frac{1}{3000}$ "
Thickness of ditto,	$\frac{1}{4000}$ "
Length of fibre-cell, 13,	$\frac{1}{750}$ "
Breadth of ditto,	$\frac{1}{1250}$ "
Longitudinal measurement of nucleus of ditto,	$\frac{1}{5500}$ "
Transverse, ditto,	$\frac{1}{3500}$ "
Length of fibre-cell, 15,	$\frac{1}{1000}$ "

Hence it appears that the length of the most contracted fibre-cell is the same as that of the nucleus of an extended one. The fibres vary somewhat in breadth, independently of the results of contraction. Thus, one in the extended condition which I sketched, but which is not here shown, measured only $\frac{1}{4000}$ inch across. The nuclei of the uncontracted fibres are very constantly of the same length, and are good examples of the rod-shape to which KÖLLIKER has directed particular attention. They always possess one or two nucleoli, and have often a slightly granular character; occasionally, as in fig. 21, they present an appearance of transverse markings. One frequently sees near the nucleus of a fibre that has been artificially extended from the contracted state, an appearance of a gap in the substance of the cell, forming a sort of extension of the nucleus, as if the fibre generally had been stretched more completely than the nucleus: an example of this is presented by fig. 7. Mr ELLIS lays great stress on a dotted appearance which he considers characteristic of involuntary muscular fibre. I must say I agree with KÖLLIKER in finding the fibre-cells, for the most part, homogeneous when extended, or faintly marked with longitudinal striæ.* No doubt dots are present in abundance; but these, so far as I have observed them in the pig's intestine, are distinctly exterior to the fibres, though adherent to their surface; and I suspect them to be little globules of a tenacious connecting fluid. That the fibre-cells do stick very tightly together, may be seen by drying a minute portion of the tissue, after which they will be found shrunk, and slightly separated from one another, but connected more or less by minute threads.

To sum up the general results to which we are led by the facts above mentioned. It appears that in the arteries of the frog, and in the intestine of the pig, the involuntary muscular tissue is composed of slightly-flattened elongated elements, with tapering extremities, each provided at its central and thickest part with a single cylindrical nucleus embedded in its substance.

Professor KÖLLIKER's account of the tissue being thus completely confirmed in these two instances, and the description here given of its appearance in the arteries of the frog's foot being an independent confirmation of the general doctrine, there seems no reason any longer to doubt its truth.

* The longitudinal striæ above referred to, are probably due to a fine fibrous structure in the substance of the fibre-cells. When in London, last Christmas, I had, through the kindness of Dr SHARPEY, the opportunity of examining a specimen of muscle from the stomach of a rabbit, which he had prepared after REICHERT's method. The nitric acid had not only detached the fibre-cells from one another, but also brought out very distinctly in each muscular element the appearance of minute parallel longitudinal fibres, which seemed to make up the entire mass of the fibre-cell except the nucleus. In a plate accompanying the paper on the Iris, before referred to, I gave figures of some fibre-cells with distinct granules arranged in longitudinal and transverse rows. This appearance, which, however, so far as my experience goes, is exceptional, and is hardly sufficiently marked to deserve the appellation "dotted," is probably caused by unequal contractions in the constituent material.—2d April 1857.



(The Figures all magnified 560 diameters except Figs. 17. 18.)

It further appears, that in the pig's intestine the muscular elements are, on the one hand, capable of an extraordinary degree of extension, and, on the other hand, are endowed with a marvellous faculty of contraction, by which they may be reduced from the condition of very long fibres to that of almost globular masses. In the extended state they have a soft, delicate, and usually homogeneous aspect, which becomes altered during contraction by the supervention of highly refracting transverse ribs, which grow thicker and more approximated as the process advances. Meanwhile, the "rod-shaped" nucleus appears to be pinched up by the contracting fibre till it assumes a slightly oval form, with the longer diameter transversely placed.

I will only further remark, that these properties of the constituent elements of involuntary muscular fibre explain, in a very beautiful manner, the extraordinary range of contractility which characterizes the hollow viscera.

EXPLANATION OF PLATE XV.

- Fig. 1 represents part of a fibre-cell from the pig's intestine, drawn out into a very fine thread.
 Figs. 2 and 3, fibre-cells from the same situation, considerably extended.
 Fig. 4, fibre-cells exhibiting faint longitudinal striation.
 Figs. 5, 6, and 7, fibre-cells imperfectly contracted.
 Figs. 8 and 9, small fibre-cells considerably contracted.
 Figs. 10, 11, 12, 13, 14 and 15, fibre-cells extremely contracted.
 Fig. 16, a fibre-cell curled up, showing the position of the nucleus embedded in its substance.
 Fig. 17, part of a moderately contracted fasciculus of unstriated muscle from the pig's intestine, as seen under a rather low magnifying power.
 Fig. 18, a small artery from the frog's web, under a rather low magnifying power.
 Fig. 19, part of the same vessel highly magnified, showing the spiral arrangement of the muscular fibre-cells.
 Figs. 20 and 21, muscular fibre-cells from another artery. In fig. 20, the spirals are much closer than in fig. 19; and in fig. 21, the spiral is quite close.
 Figs. 22 and 23 represent some fibre-cells in arteries of extreme minuteness, and show the section of the nucleus surrounded by that of the fibre-cell.