VII. On the Development of Striated Muscular Fibre. By Wilson Fox, M.D. Lond., Professor of Pathological Anatomy at University College, London. Communicated by Dr. Sharpey, Sec. R.S.

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A correct knowledge of the structure of the animal tissues has long been recognized as an almost essential preliminary to a full comprehension both of their physiological functions and also of the phenomena presented by disease, and the aid afforded in an inquiry into adult structure by the study of the processes of embryological formation has been fully appreciated.

While, however, the mode of growth of most of the tissues in question has been more or less completely elucidated, the development of striated muscular fibre has still remained a subject of considerable uncertainty, regarding which the most varied and even opposing views have been brought forward by observers who have made it an object of research. Feeling desirous, on pathological grounds, of attaining to some fixity in my own opinions on this question, I have during some months made it a subject of renewed study, and with results, which may, I venture to hope, assist at least to an elucidation of some of the points in dispute.

My observations have been conducted upon the Tadpole, Chick, Sheep, and human embryos at various stages of growth; and I hope to be able to show that the processes observed are essentially identical in all these classes.

Tadpoles (which offer by far the easiest objects for this investigation) are best examined immediately after they have emerged from the egg. The examination is much facilitated by placing them for a few hours in an extremely dilute solution of chromic acid  $(\frac{1}{8}$  to  $\frac{1}{12}$  per cent.), or (what is far better) in solutions of bichromate of potash of from 1 to 4 per cent. They should subsequently be immersed in Beale's carmine and glycerine solution\*, which renders the nuclei more apparent. The embryo chick is also best examined after immersion for a day or two in weak solutions of bichromate of potash (chromic acid is unsuitable for these preparations), and subsequent staining with Beale's solution. Sheep larger than  $\frac{1}{2}$  an inch may be examined after preparation in chromic acid of from  $\frac{1}{8}$  to 1 per cent.; at earlier stages either bichromate of potash or a mixture of alcohol, glycerine, and water forms a good medium; but in all instances I have found Beale's carmine an excellent help in this investigation. The structure of the early stages of the muscular fibre of the Tadpole may be investigated with a magnifying power of

\* How to Work with the Microscope, p. 201.

MDCCCLXVI.

600 diam. linear. I have, however, found that it is best to use a power of 900 for this purpose, Powell and Lealand's  $\frac{1}{12}$  object-glass with eyepiece No. 2 giving a much clearer view of many points than can be obtained with lower powers.

For the proper investigation of the earliest stages in the Chick and Mammalia I regard a power of at least 900 as essential, and many points can only be satisfactorily elucidated with a power of 1250 or 1850 diam. lin. I have used for this purpose a  $\frac{1}{25}$  object-glass of Powell and Lealand's, respecting the value of which I can endorse all that has been said in its favour by Dr. Lionel Beale.

In the Tadpole, immediately after quitting the egg, there will be found at the extremity of the tail muscles in all stages of development. These are represented in Plate V. figs. 1 to 9.

The earliest forms which indicate any differentiation from the round cells of the embryo are indicated by the appearance of oval bodies measuring from  $\frac{1}{500}$  to  $\frac{1}{800}$  of an inch in length with a breadth of  $\frac{1}{1000}$  to  $\frac{1}{1500}$ . They contain a clear oval nucleus, measuring  $\frac{1}{3000} \times \frac{1}{2000}$  of an inch in its longer and shorter diameters respectively. The remainder of this body is densely filled with black pigment-granules and glistening scales and masses, regarding which I have no further observations to offer.

I am disposed to term these bodies cells, not that I have been able to see around them a well-defined membrane, such a structure not becoming apparent until a somewhat later stage of their development; but their outline is so sharply defined, they alter their shape so little under moderate pressure, and form such distinct isolated anatomical elements, that I believe that a wall must exist around them even at this earliest stage, especially as one can be proved to exist at a period very little later in their development, and to which the transition only takes place by insensible gradations\*. As this, however, is still a subject of considerable discussion among anatomists †, I can only give these reasons for my opinion with considerable diffidence. I have never observed any earlier stages of these bodies, nor any appearances of the building up of granular matter around a nucleus, and I believe them to result from the first differentiation of the round formative cells of the embryo,—bodies around which there is an almost equal difficulty in proving the existence of a cell-wall, but which maintain their individuality and uniformity of size and structure both under the condition of mutual pressure, and also when artificially These structures, which with the above explanation I shall, for convenience sake, call cells, then elongate, so as to attain a considerable length without any necessary alteration in the apparent size of the nucleus, which ordinarily maintains a central position in relation to the long diameter of the cell; but laterally, as seen in profile, it

<sup>\*</sup> On reexamining some of my preparations after preservation for twelve months in strong glycerine, I find that in some of these early cells the contents have shrunk, and that a membrane has become quite distinct around considerable portions of their outline.—June 6, 1866.

<sup>†</sup> See especially M. Schulze, "Ueber Muskelkörperchen und das was man eine Zelle zu nennen habe," Reichert und D. B. Reymond's Archiv, 1861.

is often observed to be situated near the border of the transverse diameter. In other cases (Plate V. fig. 2) the nucleus appears nearer to one extremity; sometimes it may be considerably elongated (as if showing a tendency to commencing division (Plate V. figs. 3 & 4)), and occasionally opposite to it there may be a bulging in the outline of the cell.

On the other hand, the elongation of the cells may be accompanied throughout by a multiplication of the nuclei contained in their interior, giving rise to forms such as are represented in Plate V. figs. 6, 7, 8, 9. By far the most ordinary condition which I have seen, has been that the long diameter of the nucleus lies parallel to that of the cell; but in a certain number of cases, at least in those where two or more nuclei have existed in its interior, they have been placed with their longer axis transverse to that of the cell (see Plate V. figs. 8, 9).

When the primary muscle-cell, whether with or without multiplication of its nuclei, has thus become elongated to a certain extent\*, a change in its structure becomes Usually at one side a part appears lighter than the rest, and in this position sometimes a longitudinal, sometimes a transverse striation makes its appearance, or occasionally both longitudinal and transverse striation appear simultaneously. between this portion (which when seen in profile is observed along the entire border of the cell) and the dark pigmented condition of the remainder of the cell-contents is so great as in some cases to lead almost to the supposition that it is a band of striated matter laid in apposition with and external to the cell; and this is especially the case in the very earliest stages, when the altered part, as seen in profile, appears extremely narrow. I measured it (Plate V. fig. 9) at a period when it was only  $\frac{1}{25000}$  inch in transverse diameter. At a later stage (as in Plate V. figs. 5, 8, & 10) it will be seen that the change is one really affecting the cell-contents; there is no sharp line of demarcation between the altered and unaltered portions, and over the former grains and granules of pigment are seen scattered, but to a much less degree than is observed in the remainder of the cell-contents.

Gradually the pigment diminishes in the cells, and they may then be said to be separable into two portions, one striated longitudinally and transversely, the other granular, and more or less pigmented, and in this latter portion one or more nuclei are contained. At this stage a distinct membrane may be seen bounding the granular portion, as in Plate V. fig. 11. I believe that in the cases where the membrane can only be distinguished on one side of the structure, its apparent absence on the other is only the result of the position in which the object is seen; for if the membrane (which is very thin and delicate) lies in close apposition to the striated portion, its separate outline will be quite undistinguishable; and I am confirmed in this belief by the fact, that often in the same preparation, and at stages not much further advanced, cells can be found on which a membrane can also be seen on the other side of the striated portion (when seen in

<sup>\*</sup> I have, to avoid repetition, given the measurement of these figures in the special description of them at the end of this paper.

profile), and separated from it by a distinct space which is filled with the same granular and pigmentary matter as that which occupies the portion of the cell which is free from striation, and which may be seen, on altering the focus, to be diffused, but much less thickly, over the surface of the striated part; so that, to my own mind, the conclusion is unavoidable, that the membrane, which can be thus distinctly seen in profile at the edges of the fibres, encloses a space occupied (1) by one or more nuclei, (2) by a substance striated both longitudinally and transversely, and (3) by amorphous transparent matter containing suspended in it a varying amount of granules and pigment. Sometimes a nucleus may be seen on each side of the striated portion, in which case the membrane and granular contents can be seen to extend continuously over the whole structure (see Plate V. fig. 13).

In some cases, when a muscle-cell at an early stage of its development contains two or more nuclei, it shows a tendency to division, which is evidenced by an imperfect constriction between the nuclei, this constriction being apparent on that side only of the cell which contains the nuclei, and not affecting the striated portion, if the latter change has already made its appearance within the cell. At later stages, when the fibre has become more elongated, and the nuclei are further removed from each other, there is always a depression in the outline of the membrane between the nuclei, and this may sometimes proceed to a considerable depth, but never, as far as I have observed, to a complete constriction and separation between the different nuclei (see Plate V. figs. 9, 10, 14, 16).

There is a considerable difference in the number of the nuclei contained within the membrane. They may sometimes be very numerous, and the breadth of the fibre is usually proportioned to the number of the nuclei. At other times a long cell may contain only two or three nuclei; and the difference appears to depend on the multiplication of the nuclei occurring at variable periods in the development of the fibre. Thus figs. 8, 9, 10, and 17 illustrate progressive stages of this process in those rarer cases in which the nuclei are found lying across the long diameter of the fibre; while figs. 7, 11, 12, 14, and 16 are instances of fibres, or portions of fibres where many nuclei exist within the membrane.

On the other hand, during the early stages of development, instances are found, with even greater frequency, in which a very long fibre, much narrower than the others, has only a single nucleus attached to it throughout its whole course (Plate V. figs. 18 & 19 represent front and profile views of this condition). With a high magnifying power it may in these cases be distinctly seen that a delicate membrane, often separated somewhat from the nucleus by granular contents, limits the outline of the fibre, on which its prolongation can often be traced to some little distance from the nucleus.

These fibres often lie imbricated closely together, and in their earlier stages they may often be seen presenting a spindle-shaped appearance (like Plate V. figs. 24 & 25, from the Chick); by which I think that the inference is further justified that these fibres may be regarded as having been produced by the continuous elongation of cells such as are

represented in Plate V. figs. 3, 5. In one instance, represented in Plate V. fig. 20, I found a fibre dividing, and I could distinctly trace a delicate membrane outside the nuclei which lay on the fibre contained on each of the divisions\*.

As development advances, the amount of space occupied relatively by the granular portion of the contents of the membranous envelope diminishes, and its place becomes gradually occupied by striated matter. A membrane may still be traced over the whole structure; and in all the instances which I examined, the nuclei were situated between the membrane and the striated portion, surrounded by a little dimly granular material. Plate V. fig. 21 represents this condition from the upper part of the tail of a Tadpole one week old, when, though seen in profile, it will be observed that the structure has all the characteristics of adult muscular fibre.

The investigation of the earlier stages of muscle in the Chick presents much greater difficulties than in the Tadpole, owing both to the smaller size, and also to the extreme delicacy of the structures concerned.

The first differentiation from the round formative cells of the embryo which I have observed commences from the second day of incubation. At this period there are found in the dorsal region oval bodies such as are depicted in Plate V. figs. 22, 23, fig. 22 measuring  $\frac{1}{1190}$  of an inch in length by  $\frac{1}{5555}$  of an inch in breadth, and containing a nucleus (in which a nucleolus is often but not invariably seen) of  $\frac{1}{6000} \times \frac{1}{7000}$  of an inch.

These bodies are dimly nebulous; they have a distinct, clear, well-defined outline, but they cannot be seen to be surrounded by a membrane. The nucleus is often granular, and its outline is particularly well defined.

Almost simultaneously (i. e. after forty-eight hours of incubation), appears a series of forms like figs. 23, 24, 25 (Plate V.)—bodies tending to become fusiform, and of which I believe fig. 23 to represent the earliest stage. These also have in most cases a well-defined outline, and contain a large nucleus which is particularly well defined (Plate V. figs. 24 & 25 represent the appearances most commonly seen at this date, fig. 25 being a similar structure to fig. 24, but seen in profile). For the same reasons as I stated with regard to similar structures in the Tadpole, I look upon these bodies as cells; and the impossibility of distinguishing any isolable membrane arises, I believe, only from its excessive tenuity. They gradually tend to become much elongated and tapering at their extremities, and at this stage form a continuous tissue (as seen at Plate V. fig. 26), in which, though these bodies lie in close apposition, the outlines of each are distinctly maintained. In some of these bodies at this stage, two nuclei are seen (Plate V. fig. 27), but this does not appear to be the rule in the Chick.

Even at this early stage there may sometimes be seen a faint striation of portions of the cell-contents, usually appearing first in the form of longitudinal striæ (as seen in Plate V. fig. 25–27, figured in profile).

By the commencement of the third day of incubation a further change has appeared in

\* Kölliker has also seen a division of the fibres in the tail of the Tadpole, but does not mention whether he observed the membrane thus disposed (Gewebelehre, p. 102).

these bodies, as shown in figs. 29 & 30 (Plates V. & VI). They have become much elongated, forming long fibres, in which both a longitudinal and often a transverse striation are apparent; and towards the centre of the long axis of each of these fibres appears the nucleus, external to which is a distinct membrane, which can often be seen prolonged upon the fibre for some distance. These fibres form a dense tissue, the enlargements opposite the nuclei fitting into the narrower portions of adjacent fibres. Sometimes intermediate stages are seen between these and the earlier spindle-shaped cells (as in Plate V. fig. 28).

From the fourth to the fifth day a further change in the fibres is observed, which consists in the multiplication of the nuclei in their interior (see Plate VI. figs. 31-34).

At this stage the presence of a membrane enclosing the nuclei is very apparent. The nuclei form groups of two, three, four, or more in number, and vary somewhat in size. They are seen to have very distinct nucleoli, and are surrounded on all sides, except where they lie in apposition with the striated portion, with a granular nebulous matter, which may often be seen contained under the membrane, surrounding the narrower portion of the fibre. Figs. 31 & 32 (Plate VI.) are profile and front views of fibres, with a group of nuclei situated upon them and enclosed by a membrane which, though of considerable tenuity, is sufficiently defined to give a sharp double contour to the fibre. At fig. 34 (Plate VI.) the nuclei may be seen lying in various positions on the fibre, enclosed by a very distinct membrane, which at  $\alpha$  is thrown into folds across the surface of the fibre. After the fifth day the multiplication of the nuclei proceeds to a much greater extent, and is attended with an increase in thickness of the striated portion of the fibre. Thus figs. 36 & 37 (Plate VI.) represent fibres from a Chick at the seventh and eighth days, as seen in front view and profile, and in which the conditions are similar to those previously observed, with the exception of such differences as arise from the increase in thickness of the fibre. The continuity of the external membrane is very noticeable, especially when it extends over the bulgings created by the groups of nuclei.

I have found the process of development in the Sheep to proceed in precisely the same manner as in the Tadpole and Chick. Figs. 38–44 (Plate VI.) represent forms which I have seen, and which are almost precisely identical with those seen in the Chick. The process of early growth can be best observed in the budding extremities of embryos of half an inch in length; but the multiplication of the nuclei, as seen in fig. 44, proceeds until the embryo is 4 or 5 inches long.

The earliest period at which I have examined the human embryo was at six weeks. I then found structures like figs. 45 & 46 (Plate VI.), which will be seen to correspond in all particulars to figs. 18 & 19 (Plate V.), from the Frog, figs. 29 & 30, from the Chick, and figs. 39 & 40, from the Sheep. I have not succeeded in obtaining embryos between the sixth week and the third month. By the latter period the muscles have for the most part acquired their adult structure, though the fibres are very much narrower.

The development of the muscular structure of the heart differs in some remarkable particulars from those of the extremities. Three points are noticeable in the fibres of the fully-formed heart:—1, the small size of many of the fibres; 2, the apparent absence of a sarcolemma; and 3, anastomoses between and occasional branching of the fibres.

If the heart of a Chick be examined after twenty-four hours of incubation, it will be found to consist chiefly of round cells, a few fusiform having made their appearance, and some stellate cells being also present. The stellate cells become more numerous after forty-eight hours of incubation, and by the third day large tracts of the tissue of the heart are seen to consist of these cells, which anastomose freely with one another in all directions\*. Fig. 47 (Plate VI.) represents these cells at this period. They are most irregular in size and shape, giving off processes in all directions, which are of very variable thickness, and which often unite with those given off from other cells. Their contents are very granular, and there are some very indistinct appearances of striation in them. Their outline is very sharply defined (at least after the use of a solution of bichromate of potash), and around many of them I could trace a distinct double contour. Their nuclei also are very variable in size, and very irregular in position, sometimes having a situation nearly central in the cells, in other places occurring in groups upon the prolongations (b, fig. 47, Plate VI.).

By the fourth day they form a more continuous tissue (Plate VI. fig. 48), interlacing in all directions and giving rise to an extremely complex structure, composed of trabeculæ crossing one another in all directions, in which, in parts, the origin from the earlier stellate and anastomosing cells can still be seen. The nuclei are scattered very irregularly over these structures. The trabeculæ are all at this period very distinctly striated, the striation being more marked in the longitudinal than in the transverse diameters, and the tissue is extremely granular.

The increasing complexity of structure renders it difficult to follow with any certainty the further development of this structure after the fourth and fifth days of incubation; but from the fifth to the eighth day there appear, in addition, numerous elongated bodies, sometimes single, sometimes divided at their ends, and containing one or more nuclei, dimly granular throughout, and having at these periods no traces of striation, and presenting in the majority of instances (except when divided at their ends) the strongest possible resemblance to organic muscular fibre. They appear to be unconnected with the interlacing network formed by the stellate cells last described, but I have not been able to trace their further destination.

## Conclusions.

It will be seen from the foregoing description that I regard the development of mus-

\* These are too numerous, and occupy too large a relative amount of tissue, to be concerned simply in the formation of nervous ganglia; moreover their subsequent development shows them to be early stages of muscular fibre.

cular fibre as a process which consists in a definite series of changes, commencing in the cells of the early embryo. I have already adduced the reasons which dispose me to retain this term for these very distinct anatomical structures, and in their later stages I regard them as possessing all the essential elements of a cell\* (see Plate V. figs. 6-13, from Tadpole). This, however, is a question on which the opinions of different observers seem at present scarcely reconcileable, and therefore it is only possible for me here to state my individual views as to their nature. The next most noticeable feature which is to be observed is the change which takes place in the interior of these bodies,—a portion of their substance undergoing a conversion into a material which has all the recognized visible characters of muscular tissue, while the nucleus continues free and external to the striated portion, the whole being surrounded by a membrane. This membrane I regard as the sarcolemma, as its presence can be traced with high magnifying powers through every gradation of development, from almost the earliest stage, to that of fully-formed muscle; and I am therefore disposed to regard it as produced by an extension of growth, including an increase in thickness of the outermost layer (whether or not this be termed cell-wall) of the primitive cell. brane is not demonstrable in the fibres of the heart at later stages, may, I think, possibly be due to its exceeding tenuity rather than to its absence; and as the position of the nuclei in relation to the fibre is similar to that found in other muscular tissue, it appears to me probable that the whole structure is held together by some limiting wall; since it is seen that in all stages of development the nuclei are external to the striated portion, which appears to be formed by a gradual conversion of fresh material accumulated during growth within the sarcolemma, and probably under the influence of the nuclei as nutritive centres. These latter seldom or never appear to be imbedded within the fibres, though here and there, in the muscular fibre of the adult Frog, this may occasionally be the case.

It will be seen that my descriptions accord very closely with those of Lebert, Remak, and Kölliker. The early stages in the Chick and Tadpole (Plate V. figs. 2–6, & fig. 22) correspond very closely with M. Lebert's figs. 11, 21, & 26†. After this stage there is, however, some discrepancy between M. Lebert's and my descriptions, as there is also in the period at which he states the differentiation in the Chick to commence. I have found it most distinctly at the end of forty-eight hours of incubation in the dorsal region; M. Lebert states that no traces of muscular fibre are formed before the fifth day,—a period at which I have found the process considerably advanced. M. Lebert's description of the development of the fibres of the heart differs considerably from mine.

My observations correspond closely with those of Professors Remart and Kölliker §, and I am glad to know that the opinions at which I have arrived agree with those

<sup>\*</sup> See note, p. 102. † Ann. Sciences Nat. 3rd series, vol. ii. 1849.

<sup>#</sup> Froriep's Notizen, 1845; and Entwickelung der Wirbelthiere, Taf. xi.

<sup>§</sup> Zeitsch. Wiss. Zool. ix. Gewebelehre, 1862.

which have been maintained by these distinguished observers. There is, however, one point on which I would venture to express an opinion differing somewhat from that held by Professor Kölliker, though the question is of comparatively minor importance; and instead of regarding, with him, each fibre as a single many-nucleated cell, I should be disposed to consider it as a structure representing a series of many potential cells\*, which have, however, never separated, their division after the multiplication of their nuclei having been prevented by the fibrillation longitudinally (as shown by striation) of a portion of their contents, while the sarcolemma would thus represent the united membranes of many cells which have been engaged in the formation of the fibre. view may suggest that of Schwann's t, of which it may be regarded as a modification; but the differences between his descriptions (in which he represents a fibre as formed by the fusion of a series of preexistent and independent cell-structures) and mine will be at once apparent. Of course, when a fibre, as in the early stages, contains only one nucleus, it must then be regarded as a single cell. With regard to the opinions entertained by MARGO I, that the growth of muscular fibre is due to the fusion of spindle-shaped cells, it is one which for a long time I was disposed to entertain; and figures may often be obtained in breaking up the fibres of sheep of 3 or 4 inches in length, after hardening in chromic acid, which would seem strongly to support this view. Such appearances are, however, I believe, due to three causes. In the first place, fibres in very different stages of development lie frequently in close apposition, and when partially separated, the less mature fibre may often look like a portion of a broader and more fully formed one. Secondly, under the same circumstances nuclei with a portion of the sarcolemma may be detached from the surface of the fibre, and, when hanging from it, may present Thirdly, capillaries developing on the a great resemblance to spindle-shaped cells. external surface of the fibres have a most deceptive resemblance to long spindle-shaped cells, into which indeed they can sometimes be broken up. It also militates very strongly against this view that, in tearing up adult muscles hardened in chromic acid, the nuclei are never found in connexion with the fibrillæ, but are always separated as distinct bodies; often with a little granular matter around them, the remains of the original cell-contents by which they are surrounded within the sarcolemma. spindle-shaped bodies or anastomoses from processes given off from the nuclei within the sarcolemma which have been described by some later observers §, I have seen nothing either in adult or in fœtal muscles.

It would, however, be out of my province in this place to attempt a criticism of the labours of the very numerous writers || upon this subject, or to show wherein the views

<sup>\*</sup> Though this may appear almost a truism, yet I think it a consideration of some importance in relation to some of the pathological processes affecting muscle.

<sup>†</sup> Microscopical Researches, Syd. Soc. Trans. p. 137. 
‡ Quoted by Kölliker, Gewebelehre, ed. 1863.

<sup>§</sup> Leydig, Müller's Arch. 1856; Welcker, Zeit. Rat. Med. viii.; Boettcher, Virch. Arch. xiii.; Sczelkow, Virch. Arch. xix.

<sup>||</sup> See Kölliker's Gewebelehre, ed. 1862.

which I have been led to entertain agree with or differ from theirs; but I think it may be desirable for me to refer to two papers which have already appeared at the meetings of this Society, and in which the views expressed by their authors are somewhat at variance with those which my observations have led me to form.

Mr. Savory's\* observations were made on the dorsal muscles of embryos at a period when their development is already considerably advanced. It will be seen that my figures of the Chick and Sheep in more advanced stages correspond very closely to his drawings, though I have not thought it necessary to multiply figures of the enlargement and maturation of the fibres, as these have been already most ably depicted in the paper in question.

There is one other observer, whose distinguished position entitles his observations to the greatest respect, but with whose views my own cannot be brought to correspond. I refer to Mr. Lockhart Clarke, who, in vol. xi. of the 'Proceedings' of this Society', has advanced the view that the development of the fibre proceeds by a fibrillation of blastema There is at once a discrepancy between Mr. Clarke's observations and upon free nuclei. mine as to the period at which a distinct differentiation of muscular tissue occurs, he placing it at the fifth day of incubation, while I have found it distinctly advanced after Mr. Clarke regards such figures as 24 & 25 in my Plates as the forty-eight hours. result of the deposition of granular matter around a nucleus, the granular matter forming a fibre and embracing the nucleus; I regard them as the further elongation of such cells as figs. 22 & 23 represent. I have already given my reasons for regarding these structures as possessed of a sharp limiting outline, though, of course, in breaking up such delicate structures for microscopic examination, free nuclei surrounded by more or less granular matter will frequently be more numerous objects in the field than the complete cell-forms, which are often comparatively rare and difficult to find, but which I regard, as will be seen from the foregoing, as the essential element in the development of these structures.

## DESCRIPTION OF THE PLATES.

## PLATE V.

Figs. 1-21 represent the development of muscular fibre in the Tadpole, × 900 diameters.

- Fig. 1. First appearance of muscular tissue at extremity of tail of newly-born Tadpole. Outline well defined, though membrane not apparent. Measures  $\frac{1}{1000}$  of an inch in length,  $\frac{1}{2000}$  of an inch in breadth. Nucleus measures  $\frac{1}{3000} \times \frac{1}{5000}$  of an inch.
  - \* Philosophical Transactions, 1855.
- † Also in Microscop. Journ. 1862.

- Figs. 2-5 are further stages of the elongation of fig. 1; fig. 5 measured  $\frac{1}{450}$  of an inch in length,  $\frac{1}{1000}$  of an inch in breadth. Their nuclei averaged  $\frac{1}{1000}$  of an inch in length,  $\frac{1}{3000}$  to  $\frac{1}{5000}$  of an inch in breadth. In fig. 4 a faint appearance of striation is becoming visible, which is much more apparent in fig. 5, where it can be seen that the striation passes by insensible gradations into the contents of the rest of the cell.
- Fig. 6-10 are cells with many nuclei. In figs. 8, 9, & 10 striation is already appearing.
  Figs. 11-13 are cells whose pigment has partially disappeared, and around which a membrane is distinct. The membrane is best seen on the striated side of fig. 11 (seen in profile). The contents of the cells are seen to be differentiated into a granular portion and a striated. The nuclei are found in the former. Length of fig. 13, <sup>1</sup>/<sub>410</sub> of an inch, breadth <sup>1</sup>/<sub>1000</sub> of an inch; breadth of central band, <sup>1</sup>/<sub>5000</sub> of an inch; length of nuclei, <sup>1</sup>/<sub>2000</sub> of an inch; breadth of nuclei, <sup>1</sup>/<sub>3600</sub>
- Figs. 14–16 show portions of fibres in more advanced stages of same process. Total breadth of fig. 16,  $\frac{1}{2400}$  of an inch; breadth of striated portion,  $\frac{1}{4000}$  of an inch. The membrane with granular contents within it can be traced over the whole of this latter fibre.
- Fig. 17 is a further stage of figs. 8-10, where the nuclei lie transversely to the fibre.

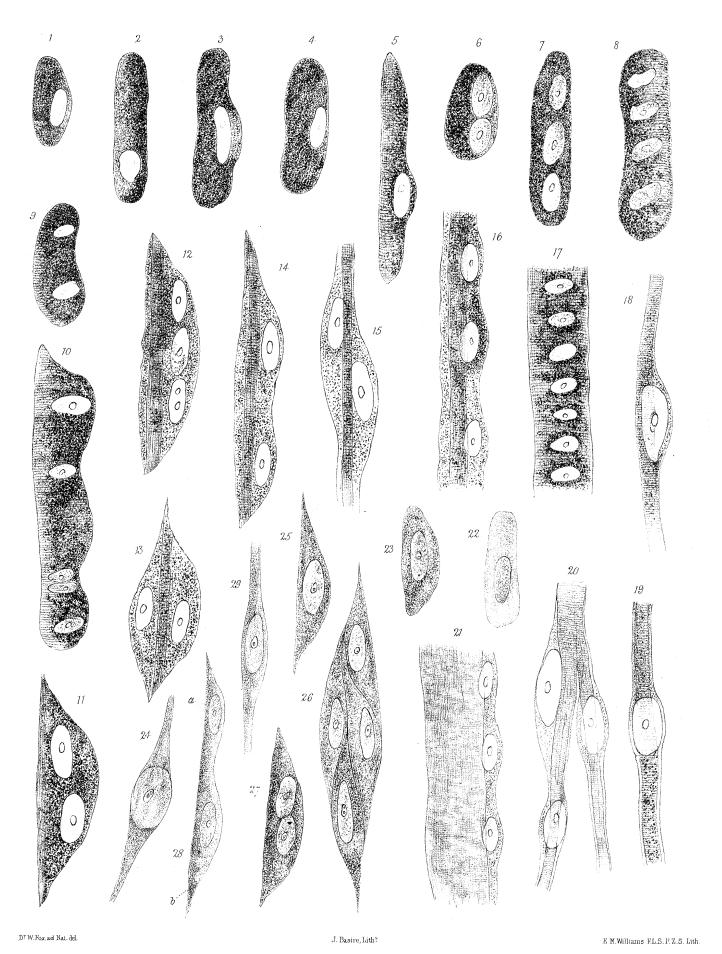
of an inch.

- Figs. 18 & 19 are front and profile views of fibres produced by the elongation of cells like fig. 5; fig. 19 is seen surrounded by a distinct double outline. Width of whole, including membrane on each side,  $\frac{1}{5000}$  of an inch; width of striated portion,  $\frac{1}{6666}$  of an inch. Such fibres may narrow at their extremities to a width of  $\frac{1}{12000}$  of an inch.
- Fig. 20. Instance where a divided fibre was found in the tail of the Tadpole; the membrane (sarcolemma) was seen continued on each division.
- Fig. 21. Fibre from the tail of a Tadpole one week old; striated portion has greatly increased in proportion to rest of contents of sarcolemma; nuclei are seen within sarcolemma, but external to striated portion. Width of whole,  $\frac{1}{1100}$  of an inch, of striated portion,  $\frac{1}{1250}$  of an inch.

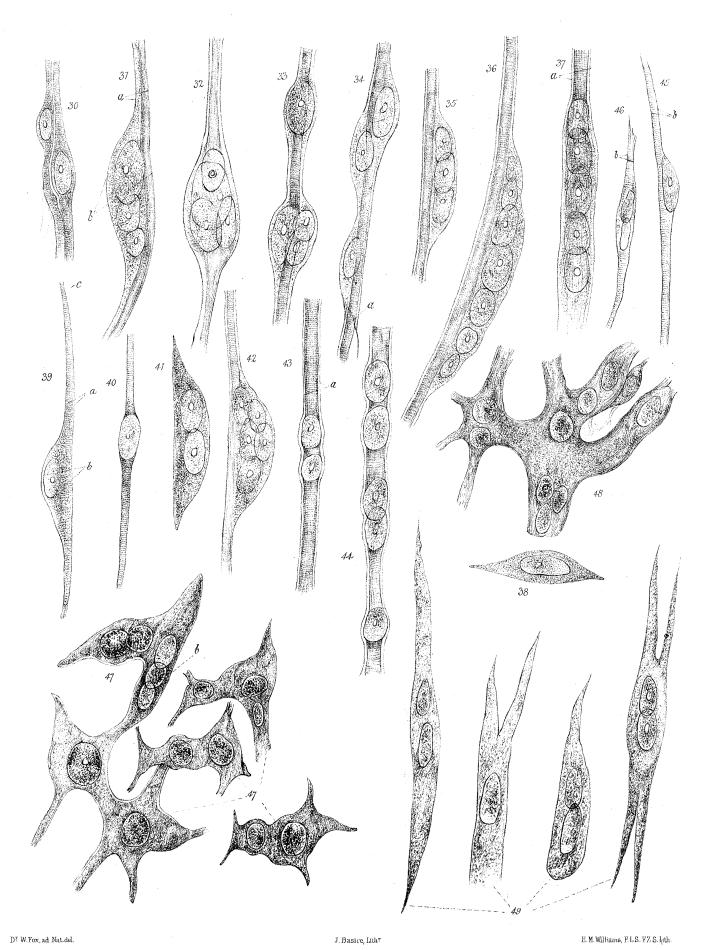
## PLATE VI.

- Figs. 22-38 represent the development of muscular fibre in the Chick×1850 diameter. Figs. 22-26 stages found after forty-eight hours of incubation.
- Fig. 22. Measures  $\frac{1}{1190}$  of an inch in length  $\times \frac{1}{5555}$  in breadth. Diameter of nucleus  $\frac{1}{6000} \times \frac{1}{7000}$  of an inch. Length of fig. 24  $\frac{1}{1500}$  of an inch; breadth  $\frac{1}{3000}$  of an inch.
- Figs. 27 & 28. From Chick after seventy-two hours' incubation. Length of fig. 28  $\frac{1}{750}$  of an inch; breadth at  $a = \frac{1}{5000}$  of an inch, at  $b = \frac{1}{12000}$  of an inch.

- Figs. 29 & 30. Chick from fourth to fifth day. The nucleus now appears prominent on the fibres with some granular matter around it, the whole being enclosed by a membrane. Width of striated portion  $\frac{1}{20000}$  to  $\frac{1}{16000}$  of an inch; width of nucleus  $\frac{1}{4500}$  of an inch.
- Figs. 31 & 32. Fibres from Chick at fourth and fifth days. Width of whole fibre (fig. 31) at  $a_{8000}^{-1}$  of an inch; width of striated portion  $\frac{1}{22000}$  to  $\frac{1}{16000}$  of an inch, width of whole enlargement at  $b_{2500}^{-1}$  of an inch.
- Figs. 33 & 34. Fibres from sixth to seventh day. Sarcolemma now very apparent. Width of striated portion of fibre (fig. 34)  $\frac{1}{12000}$  of an inch.
- Figs. 36 & 37. Fibres from Chick at eighth day. Show great increase of nuclei. Width of fibre (fig. 37) at  $a_{\frac{1}{6666}}$  of an inch.
- Figs. 38-41. From Sheep half inch in length. Length of fig.  $38 \frac{1}{1200}$  of an inch, breadth  $\frac{1}{5500}$  of an inch. Width of fig. 39 at  $a \frac{1}{3800}$  of an inch, at  $b \frac{1}{8000}$  of an inch, at  $c \frac{1}{16000}$  of an inch. Length of nucleus in fig.  $40 \frac{1}{3300}$  of an inch, breadth  $\frac{1}{4600}$  of an inch.
- Fig. 41. Shows elongation of a cell (such as fig. 38) with multiplication of its nuclei and commencing striation, and corresponds to figs. 11 & 27 from Tadpole and Chick.
- Figs. 42–44. From Sheep one inch long. Width of whole fibre (fig. 43) at  $a_{\overline{4000}}$  of an inch; of striated portion  $\frac{1}{8000}$  of an inch. The sarcolemma can be distinctly seen surrounding these preparations.
- Figs. 45 & 46. From human embryo of six weeks. These are magnified only 690 diameters, and consequently show less distinctly than those of the Sheep and Chick, the continuity of the sarcolemma outside the nucleus and fibre. Width of fibre (fig. 45) at  $b = \frac{1}{20000}$  of an inch; of nucleus  $\frac{1}{7000}$  of an inch. At fig. 46 is seen a commencing multiplication of the nuclei within the sarcolemma.
- Fig. 47. Stellate cells from heart of Chick between second and third day. Around some a double contour can be seen. ×1850 diameters.
- Fig. 48. Anastomosing fibres from heart of Chick between fourth and fifth days, ×1850 diameters.
- Fig. 49. Spindle-shaped cells from heart of Chick from fourth to eighth days.



Development of Striated Muscular Fibre.



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