XXVII. *On the Distribution of Nerves to the Elementary Fibres of Striped Muscle.* By LIONEL S. BEALE, *M.B., F.R.S., Professor of Physiology and of General and Morbid Anatomy in King's College, London; Physician to King's College Hospital.*

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No branch of minute anatomy has received a larger share of attention than the anatomy of striped muscle, and probably no one point has been more carefully investigated than the distribution of nerve-fibres to this important tissue. Very different conclusions have been arrived at, and the various questions at issue have not yet been determined satisfactorily. For the different views entertained with reference to the mode of termination of nerve-fibres I must refer to the treatises on minute anatomy, and especially to Professor KÖLLIKER'S work just published, where a summary of the results of numerous investigations will be found.

KÜHNE'S *Observations.*

The most recent observations are probably those of KÜHNE, who states that the nerve-fibre can be traced up to the sarcolemma. He concludes, with some other observers, that in the muscles of insects the axis cylinder of the nerve-fibre penetrates this transparent structure, and is connected with the rows of nuclei which are imbedded in the substance of the muscular fibre and lie amongst the fibrillæ*. As will be observed by reference to KÜHNE'S drawings, these points are very indistinctly, and, if I may so say, diffidently represented. Like KÜHNE himself, I have quite failed to demonstrate in vertebrate animals the arrangement he described in insects. It may be remarked that nuclei amongst the fibrillæ are very abundant in some fishes and reptiles (especially the frog) whose muscles are sparingly supplied with nerves, while in the muscular fibre of many birds and mammalia which are very abundantly supplied, not a single nucleus can be demonstrated in the interior of the fibre. It seems hardly likely that the relation between the nerves and the contractile elements of the tissue should be closer in these cold-blooded, and comparatively inactive vertebrata, than in birds and mammals. The nuclei amongst the fibrillæ of the muscles of vertebrate animals are clearly not connected with nerves†.

* Untersuchungen über Bewegungen und Veränderungen der contraktilen Substanzen. REICHERT and DUBOIS REYMOND'S (formerly MÜLLER'S) Archiv, 1860.

† Soon after this paper was read, I investigated the arrangement of the nerves distributed to the muscles of the larva of the common blow-fly and the perfect insect, and I have arrived at the conclusion that the nerves do not penetrate into the interior of the muscle, but are connected with some very large spindle-

General Opinion entertained.

The general opinion at the present time seems to be that nerves terminate upon the elementary fibres of voluntary muscle by free pointed extremities. KÖLLIKER considers it probable that the elementary fibres are only touched by the nerves at distant points, while some conclude that many fibres, or even parts of a muscle, are not brought into actual contact with nerve-fibres at all. As the results of my inquiries differ so entirely from those of other observers, I think it right to say that my observations have been very frequently repeated with great care, and upon several different vertebrate animals (man, dog, rat, guinea-pig, mouse, greenfinch, frog, tadpole, gudgeon, minnow, &c.). Several of the specimens from which my conclusions have been deduced are preserved permanently, and can be examined by all interested in this inquiry.

Of the Distribution of the Nerves to the Elementary Fibres.

My observations lead me to conclude that nerves are distributed to every elementary fibre of all voluntary muscles of man and vertebrate animals. The elementary fibres of some animals are much more abundantly supplied with nerves than those of others. The muscles of the mouse receive a larger supply than those of man. Generally, it may be said that the muscles of reptiles and fishes are sparingly supplied with nerves in comparison with those of mammals and birds. I have found that in the same animal a greater number of nerve-fibres are distributed to some muscles than to others. Thus the elementary fibres of the tongue of the mouse receive a larger supply than those of the diaphragm, and the latter a much larger supply than the muscles of the system generally.

The sarcolemma of the elementary fibre of the tongue and diaphragm of the mouse seem to be completely covered with nerves and vessels. The nerves for the most part run close to the capillaries, and it is not possible to separate these structures from each other without destroying them. Some of the nerve-fibres cross the elementary fibre transversely, others obliquely, while some run longitudinally on the central or lateral aspects of the fibre. In favourable specimens the nerves may be seen to divide on the surface of the sarcolemma. The ultimate fibres are, however, of such extreme delicacy, that a little careless manipulation, pressure, or a very slight alteration in the density of the fluid in which they are immersed, or in its refractive power, renders them quite invisible or destroys them altogether. I have not been able to follow an individual fibre for any great distance in consequence of the constant change in its course. These observations show that it is possible for an elementary fibre to be supplied with nerves from several different sources. It is not possible to decide, from the course of a single nerve-fibre, whether it is coming from, or passing towards, the nervous centre. Many fibres, apparently passing from one point, can be followed for some distance in opposite directions. The appearance of the nerve-fibres on the elementary muscular fibre differs

shaped cells on its surface. These are very difficult to demonstrate, but I have been able to preserve some specimens which show this point very clearly.

materially in certain cases. This seems to depend in some measure upon the state of the muscular fibre itself. If it is contracted, the nerve-fibres appear much wider than in cases where the muscle is relaxed and the fibres stretched to their full extent. The nerve-fibres undergo change very soon after death, and the finer ramifications seem to be completely destroyed by alkalies. Unless the tissue be properly preserved very soon after the death of the animal, it is not possible to demonstrate the arrangement described in this paper. The delicate structure very soon becomes destroyed by the action of air or water, and nothing is to be seen but a few oil-globules and a little fibrous-like structure.

The nerves of muscle freely divide and subdivide before they reach their ultimate distribution, as was demonstrated by WAGNER and others. I have observed that many of the ultimate fibres which appear to be single, really consist of two or more fibres in close apposition, which vary much in their diameter. Where one fibre is wide, its neighbour is reduced to a very thin cord. A little further on the diameter of the latter increases, while that of the former becomes reduced in a corresponding degree. A fibre passing into a trunk at right angles is frequently seen to divide into two branches which become lost in the trunk, but may be seen to pursue their course in opposite directions. The finest ramifications upon muscle are thin and flattened, as may be seen at points where the nerves pass round one elementary fibre to reach the surface of another.

Oval Bodies or Nuclei.

In connexion with all nerve-fibres, there are little oval corpuscles which are readily coloured by carmine. These are the nuclei of authors, and are essential constituents of the nerves, especially towards their peripheral distribution. They are soft in the recent state, and, in my preparations, have a granular appearance under the highest powers of the microscope. They contain two, three, or more distinct globules, with a clear outline and transparent centre, the so-called nucleoli. In ordinary dark bordered nerve-fibres, these bodies for the most part appear to be situated outside the white substance (nuclei of the tubular membrane); but in some nerves they may be seen in the white substance itself, and occasionally in connexion with the axis cylinder. As the nerves approach their distribution these bodies increase in number, and undoubtedly form an integral part of each separate fibre. They are often equal to the fibre in width, and sometimes they appear even wider, but this is probably due to stretching and alteration in the fibre itself. In bands composed of from five to eight or nine fibres, in the mouse, they are more than the thousandth of an inch apart, but in some of the terminal fibres they are as close as the two-thousandth of an inch. Not unfrequently four may be seen close together upon the surface of an elementary fibre.

After giving a full description of the corpuscles imbedded among the fibrillæ, as can be easily demonstrated in the large primitive fibres of the frog, Mr. BOWMAN states that in one instance he observed similar corpuscles on the exterior of a fasciculus in the chrysalis of the tiger-moth, but the relations of these were doubtful. He also noticed similar corpuscles in the sarcolemma itself*. These bodies have since been considered to belong to the areolar tissue, but it is probable that at least a certain number of them are the same corpuscles which I have described as forming an integral part of the nerve-fibre in its ultimate distribution.

I have found the oval corpuscles present in great number in nerves of sensation, as well as in those of motion, and in the nerves distributed to vessels. They are very numerous in the nerve-fibres which supply the papillæ of touch and taste. I have lately succeeded in tracing nerve-fibres amongst the muscular fibres of the heart. For the most part the bundles of nerve-fibres pass with the vessel. The compound fibres divide and subdivide, and very minute fibres may be traced for a long distance. I believe that the branches of each nerve-fibre passing into the muscular substance are brought into relation with an immense number of the muscular fibres of the heart (or rather with a considerable expanse of the muscular structure) by means of the oval nuclei, which are seen at short intervals throughout the whole course of the nerves. The distribution of the ultimate branches of the nerves to the muscular fibres of the heart is now being investigated.

The nuclei are in all probability the exact points at which all the phenomena connected with the action of nerves at their peripheral distribution occur. These bodies are more numerous in the nerves distributed to the muscles of the tongue and diaphragm of the mouse than in those of other muscles. A greater number are found in connexion with the nerve-fibres of the mouse than in those of man. Generally, the nerves of mammalia and birds have a greater number of nuclei, which are separated from each other by shorter intervals than those of reptiles and fishes. As is well known, they are found all along the trunks of the so-called gelatinous fibres, and the same disposition is met with in the nerves distributed to vessels. I have seen them on branches of the sympathetic close to their origin from ganglia, on the branches in the ganglia, and in the so-called ganglion cells or vesicles. I may be permitted to state that I have made several observations which go far to convince me that the so-called gelatinous fibres, or fibres of REMAK, are not composed of fibrous tissue merely, as is now generally believed, but are really nerve-fibres, as they were originally considered. The exact nature and arrangement of these fibres, however, require further investigation before I can arrive at definite conclusions.

The nuclei, in their general appearance, much resemble the oval corpuscles which are connected with the walls of capillaries, and which in many instances lie quite external to them; and not unfrequently, in the same situation, are also found the nuclei of fibrous tissue, those of fat vesicles and other bodies of the same character, or nearly so, the nature of which has not yet been satisfactorily determined. By some observers, all the above structures, as seen in uninjected preparations, have been called areolar-tissue-corpuscles, or connective-tissue-corpuscles (*Bindegewebe-körperchen*). The bearings of this very important question must, however, be deferred.

* Philosophical Transactions, Part II. 1840, p. 485.

I should state that the nerves and vessels may be stripped off the elementary fibre without difficulty. In this process the corpuscles external to the sarcolemma and in connexion with the nerves and capillaries are also removed. In the frog, the corpuscles which are so numerous amongst the fibrillæ remain, but in the delicate muscles of the mouse I have often observed that all traces of corpuscles are completely removed by this operation, there being, at least in many instances, none within the sarcolemma. From numerous observations I have come to the conclusion that the nerves, like the capillaries, always ramify only upon the outer surface of the sarcolemma, and do not penetrate this structure *.

Of the Manner in which Nerves terminate.

From the arrangements above referred to, it would appear that the nerves may be described as originating in a network, formed by the oval corpuscles being connected together by intermediate fibres. This network is in close relation with other elements of the tissue to which the nerves are distributed. The nerves grow as the adjacent textures grow; and in any form of fibrous tissue which has attained a certain degree of thickness, it is very difficult to demonstrate the delicate nerve-fibres running amongst the coarse fibres which interlace in all directions. They may, however, be traced out by the direction which the oval nuclei are seen to take †. The network is continually undergoing alterations by the formation of new nuclei and connecting fibres, and by the removal of old ones. From the connexion of this network with the nerve-fibres, it would seem to follow that an impression made upon a given portion of a sentient surface might be transmitted to the nervous centre by contiguous fibres, as well as by the one which would form, so to say, the shortest route; and it is possible that impulses to motion may be conveyed to muscular fibres by a more or less circuitous path, as well as by a direct one.

Of the so-called Tubular Membrane.

Nerve-fibres are imbedded in a clear transparent material, which completely surrounds large separate fibres, in the form of a membrane, as usually described (the tubular membrane of authors). In the finer divisions, however, several separate nerve-fibres, differing much in thickness, are enclosed in this structure, and when examined by a moderately high power (220 diameters), appear to form one fibre. Many such fibres, which seem to consist of a single fibre, in good specimens may be resolved by a twelfth (700 diameters) into two, three or more fibres.

Not unfrequently very narrow fibres may be traced for a long distance running close to an ordinary fibre imbedded in what would be called tubular membrane. I have seen

* It is probable that the nerves and capillaries are very intimately connected with the sarcolemma, and in some preparations one is almost led to conclude that the sarcolemma is itself composed of nerves, capillaries, and a small quantity of fibrous tissue connecting them.

† The determination of the connexions of the numerous nuclei is, as would be supposed, a very difficult matter. This difficulty, however, is greatly diminished by examining well-injected preparations, in which the nuclei connected with the capillaries can be easily distinguished from those belonging to the nerves.

branches, apparently proceeding from the axis cylinder, pass through the white substance and run parallel with the fibre for a long distance, then leave this fibre and pursue their course in close relation with another. This transparent tissue becomes much spread out as the nerve-fibres approach their distribution, and numerous delicate fibres, connected both with nerves and vessels, may be seen in it. This delicate texture might be described as a tissue in which the nerves and capillaries are imbedded. In conjunction with the nerves and capillaries, it forms an exceedingly thin expansion or membrane on the external surface of the elementary fibre. In some specimens it would appear that this is incorporated with the sarcolemma itself, but this point is doubtful. I have some preparations in which it certainly appears as if the injection had passed between this membrane and the sarcolemma, and, as before stated, I have certainly removed capillaries and nerves from the surface of the elementary fibre, leaving the sarcous material apparently invested with its sarcolemma; but this point is of course very difficult to demonstrate.

Of the Axis Cylinder and the White Substance.

I have been led to conclude that the axis cylinder divides and subdivides into many branches, so that one fibre in the trunk of a nerve may carry impressions to or from a comparatively large extent of surface. I have not yet been able to determine by actual observation if a branch of one axis cylinder may run in apposition with one or more branches from the axis cylinder of another trunk at a distance from the first, or derived from another source; but from appearances I have observed I think this possible. The axis cylinder often exhibits a tendency to split, but I have not yet been able to ascertain if it consists originally of a great many fibres which ultimately become fused into one, or of one fibre which gives off numerous branches; but from what I have seen, I feel sure that towards the distribution of the nerve the axis cylinder divides and subdivides very freely, the amount of investing substance which surrounds it (white substance of SCHWANN) becoming less and less, until in the finer ramifications it is not possible to say that a fibre consists of an axis cylinder and white substance; for its general appearance and refractive power are the same in every part, except where the nuclei are situated. The arrangement of the so-called tubular membrane has been already explained. It is not unreasonable to suppose that the definite character of the white substance of SCHWANN and the axis cylinder, in the larger nerve-fibres, results from changes which occur during the gradual growth and altered relations of the fibres which take place during the development and growth of the entire organism. The full discussion of these points demands a knowledge of the history of the development of the structures referred to. This we do not yet possess; but nevertheless I may perhaps be allowed to state briefly the conclusion to which I have arrived from investigations which, however, cannot be regarded as completely satisfactory. The axis cylinder, towards the ultimate ramifications of the nerve-fibres, loses its fibrous or band-like character, and in the ultimate subdivisions, the entire extent of the nerve-fibre possesses the same composition. There is no tubular membrane, medullary sheath or axis cylinder to be demonstrated; neither is there any definite point at which the so-called white substance can be said to cease. The axis cylinder gradually loses its hard fibrous character (frog), and the white substance its peculiar refractive power and consistence. The whole fibre, as seen in my specimens, seems to consist of a very transparent and perhaps delicately granular substance, which can be shown to be composed of fatty and albuminous material. These appearances, are, however, produced by the action of reagents (chromic acid, bichromate of potash); for in their natural state, at least with the aid of the object-glasses we possess at present, the finer ramifications of the nerve-fibres and their nuclei are quite invisible.

Of the Formation of New Fibres.

New branches are continually being developed in connexion with the terminal nerve-fibres, and a certain number of old ones appear to undergo removal. The oval bodies divide longitudinally and transversely, and the segment about to give rise to the new fibre is lengthened by elongation at each end, where the new fibre still remains in connexion with the original branch. Its nucleus divides transversely, and the length of the fibre thus goes on increasing. Traces of nerve-fibres which have ceased to perform any active office may be seen in the form of delicate fibres, which still remain in connexion with actual nerve-fibres. I am of opinion that the so-called connective tissue found in small quantity between the elementary fibres of muscle, and in some other situations, is produced in this manner. In many morbid alterations the so-called areolar, fibrous, or fibroid, or indeterminate connective tissue, certainly consists merely of the remains of other structures which have ceased to perform any active office, and cannot be completely removed by absorption.

Of Preparing the Specimens.

My specimens were obtained from preparations which had been previously injected with transparent blue injection. I found that without this operation it was not possible to follow the delicate nerve-fibres among the capillary vessels, or to distinguish these structures from each other with absolute certainty. I also adopted this plan as the only one calculated to introduce a preservative fluid simultaneously to all parts of a tissue, and with sufficient rapidity to prevent decomposition of the delicate structures under investigation. In this manner alone can alteration in chemical composition, in density and in refractive power of the preservative fluid employed, be prevented. The medium used was the same as that employed in my investigations on the anatomy of the liver* Carmine solution was used for demonstrating the arrangement of the nuclei. The exact process employed is somewhat uncertain and complicated, and is probably capable of being rendered much more uniform in its results and more simple in its application.

* Philosophical Transactions, 1856. The principles on which this fluid is employed will be found stated in the 'Microscope in its application to Practical Medicine,' p. 63, and in papers published in the first volume of the 'Archives of Medicine,' 1859, pp. 18, 152.

Conclusions.

The most important facts which have been elucidated in this inquiry appear to me to be the following:—

1. That nerve-fibres in muscle and in some other tissues, if not in all, may be traced into, and are directly continuous with, a network formed of oval nuclei and intermediate fibres.

2. That the organs by which nerves are brought into relation with other textures, and the agents concerned in the development of nerves and the formation of new fibres, are the little oval bodies or nuclei which are present in considerable number in the terminal ramifications of all nerves. A great number of these bodies is associated with exalted nervous action, while, where sparingly found, we may infer the nervous phenomena are only imperfectly manifested.

3. That every elementary fibre of striped muscle is abundantly supplied with nerves, and that the fibres of some muscles receive a much larger supply than others.

4. That the nerves lie, with the capillaries, external to, but in close contact with, the sarcolemma. They often cross the fibre at right angles, so that one nerve-fibre may influence a great number of elementary fibres. There is no evidence of their penetrating into the interior of the fibre.

Explanation of the Plate.

PLATE XXIII.

Fig. 1. Nerve distributed to the elementary fibres of a muscle from the leg of a frog. To the right, two ordinary fibres and four finer ones are seen enclosed in the same transparent tissue (tubular membrane). As these subdivide, a division occurs in this structure, but many nerves, which to ordinary powers appear to consist of a single fibre, are found really to be composed of several individual fibres enclosed in one transparent sheath. At *a* is a fibre which is widest at that part of the nerve nearest to the nervous centre, while at *b* one is represented which is wider towards the peripheral extremity of the nerve. At the lower part of the drawing, two of the fine fibres have been pressed out of the transparent tissue, and one of them is broken off.

Fig. 2. Represents a portion of a compound fibre from the tongue of the frog. The branch from this (not more than $\frac{1}{5000}$th of an inch in diameter) is seen to contain three or four separate fibres; and it will be observed that, as this nerve passes to the larger trunk, fibres proceed from it which pass in both directions,—a fact which I have repeatedly observed in all the animals I have examined.

Fig. 3. Small portion of a fine nerve-fibre from the frog's tongue dividing into several separate branches. The subdivisions of the oval bodies are well seen.

Fig. 4. Small portion of a nerve, also from the tongue of the frog, showing several fibres contained in the same transparent tissue.

Fig. 5. Portion of a muscular fibre from the diaphragm of the mouse, showing nerve-fibres and capillary vessels. Below *a* is seen a fibre connected with the nucleus close to the capillary wall, but of the nature of this and of several similar fibres I have observed in connexion with the capillary walls, I am not certain. Oval bodies are seen apparently in the capillary walls, and also external to them. Some would refer the latter to the vascular nerves, and many appearances I have observed, some of which are delineated in my drawings, favour the idea that capillaries, as well as the small arteries, are supplied with nerves; but I am not yet able to express a positive opinion on this question.

Fig. 6. From the diaphragm of the mouse, showing the manner in which the finer bundles divide and traverse the elementary fibres in all directions. The intimate relation of the nerves to the capillaries is also shown in this drawing. In nature there is a delicate tissue connecting these structures together, so that they may be readily stripped off from the elementary fibre without injuring the latter.

Fig. 7. Portion of thin elementary fibres more highly magnified. At *a*, one of the oval bodies in process of subdivision is shown.

Fig. 8. Another portion, showing how the nerves vary in diameter in different parts. In the upper part of the drawing four large nuclei are seen close together.

Fig. 9. Two oval nuclei outside a capillary connected together by a fibre, with two nerve-fibres crossing a muscular fibre.

Fig. 10. A portion of a wide muscular fibre from the diaphragm of the mouse in a state of contraction. The nerve-fibre in the upper part is seen to divide.

Fig. 11. Fine nerve-fibre from the diaphragm of the mouse, under $\frac{1}{10,000}$th of an inch in diameter, but consisting of two, if not of three or more fibres.

Fig. 12. Another appearance frequently observed.

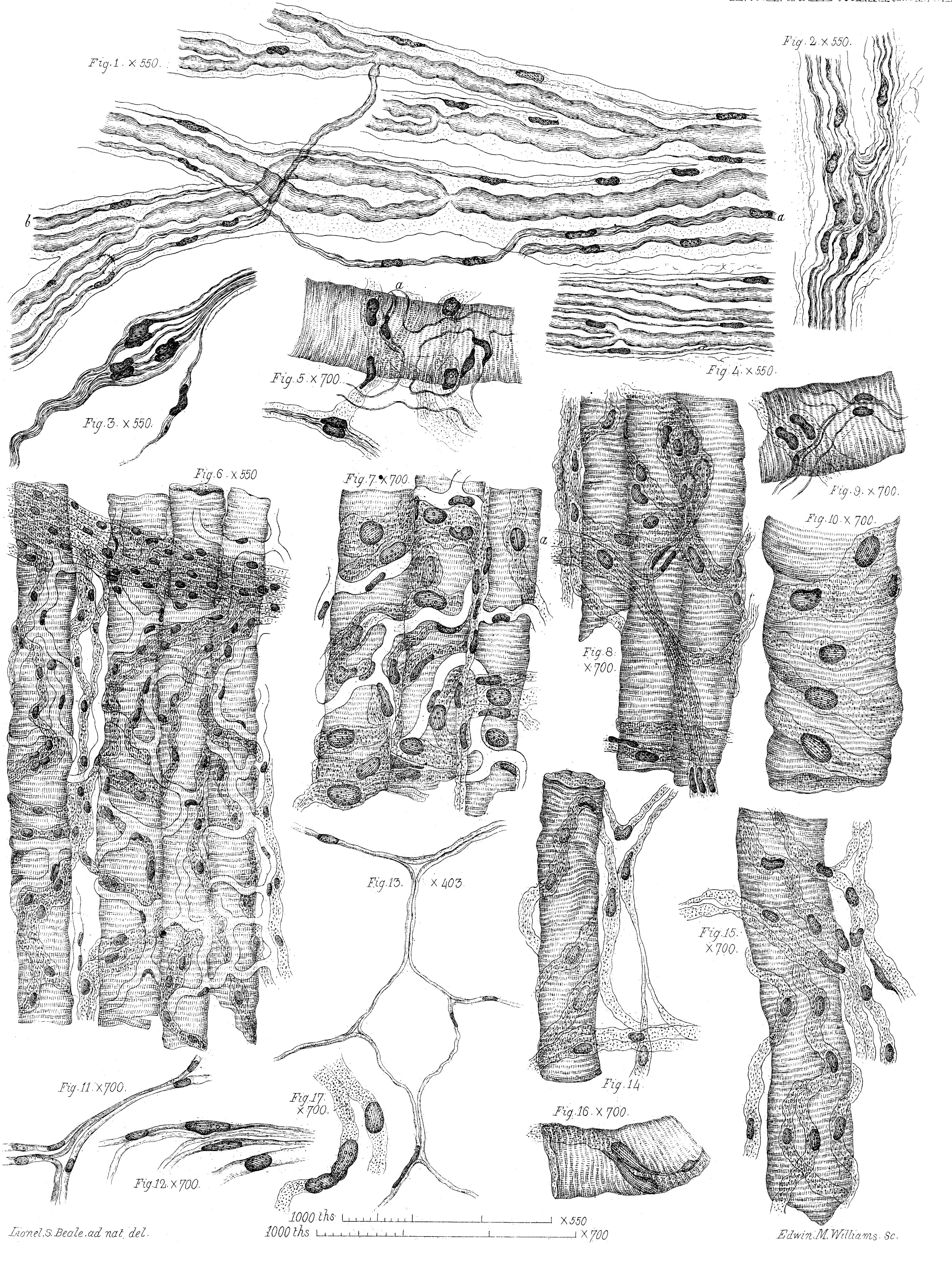
Fig. 13. Branching of very fine nerve-fibres from the tongue of the mouse.

Fig. 14. From the diaphragm of the mouse, showing the nerve-fibres between two elementary fibres of muscle.

Fig. 15. Also from the diaphragm, showing a portion of a muscular fibre nearly covered with nerves. Such specimens were common.

Fig. 16. Manner in which the oval bodies subdivide.

Fig. 17. Portion of a nerve-fibre undergoing increase in its length by the transverse division of the nucleus. From the surface of a muscular fibre from the diaphragm of the mouse.



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