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NERVE ENDINGS IN THE MUSCLES OF THE FROG

By DR N. KULCHITSKY
Anatomical Department, University College, University of London

The nerve endings in the muscles of the frog were discovered by Kühne (1861). This was the first advance made in the investigation of nerve endings in muscles after the discovery of the direct connection between nerve and muscle fibres (Doyère, 1840). From this time on, numerous investigations were carried out, which point to the interest taken by histologists in this question. It is necessary to remember, however, that Kühne's discovery was made at a time when no very precise histological methods were established and the microscope itself had hardly reached the brighter days of its development. The investigators of that time could at best use the water-immersion system, constructed for the first time by Hartnack in 1859. This is why, together with valuable discoveries which have retained their importance up to the present time, we find in histological literature a great number of observations, made by very prominent investigators, which now have only a historical importance. I venture to pass over these observations without discussion. Indeed, the period when the investigations of authors undoubtedly began to acquire a definite character, dates back to the end of the seventies or the beginning of the eighties of the last century, when the investigations of nerve endings in muscles were already being carried out by means of impregnations with gold chloride. But it was only with the introduction of vital staining of nerves with methylene blue (Ehrlich, 1886) that the investigation of nerve endings in all regions of the body was placed on a firm foundation.

The present work was done by myself according to this method and only partly by means of impregnations with gold chloride.

In the muscles of the frog the same groups of nerve fibres are distributed as in the other vertebrates:

(*a*) *Medullated nerve fibres.* These may be in the form of thick or thin fibres, depending apparently on the thickness of the myelin sheath. But, in any case, both kinds are continually encountered and Bremer, not without reason, has ascribed them to two different groups. With caution it may be admitted that both varieties of medullated fibres are provided with terminations which are not identical.

The fibres of both groups, still some distance away from their termination, divide into 2–3 and some times more branches. On reaching the muscle fibre, the terminal nerve fibres divide again more often into two branches, when they frequently lose their myelin sheath and terminate in the form of a non-medullated fibre.

(*b*) *Non-medullated nerve fibres.* These are numerous. Some of them, as we have just mentioned, result from the division of medullated fibres, diverging from these latter at the nodes of Ranvier. The others come from far away and it is difficult to say whether they have any connection with the medullated fibres or not. At least, they form extensive plexuses covering a large area and are provided with terminations differing considerably from the terminations of medullated fibres.

These non-medullated nerves belong, in my opinion, to the cerebrospinal nervous system and should not be confounded with

(*c*) the non-medullated nerve fibres of the sympathetic system, which, as has been more or less precisely established, are connected with the blood and the lymphatic vessels.

I shall now endeavour to describe the endings of the mentioned fibres according to my personal observations.

TERMINATIONS OF THE MEDULLATED NERVE FIBRES

There are two kinds. Some lie below the sarcolemma (hypolemmally), others outside the sarcolemma (epilemmally). The first are undoubtedly the terminations of motor nerve fibres, the second, in all probability, belong to sensory nerve fibres.

The relations presented by the motor nerves are quite definitely established. Every nerve fibre, after dividing several times, loses its myelin sheath, the sheath of Schwann fuses with the sarcolemma of the muscle fibre, and the axis cylinder enters into this latter and with its ramifications places itself immediately under the sarcolemma, without penetrating into the depth of the muscle substance. For a long time these relations have been handed down as a tradition by all authors, but I think in this question it is necessary to make a reservation or even perhaps more—an essential correction. One frequently comes across the statement that there enters into the muscle fibre what is described as a naked axis cylinder in the shape of a thin thread or a bundle of thinnest fibrillae. This, however, is very doubtful. The axis cylinder is never naked. On the contrary, along the whole extent of the nerve fibre, it is covered with an extremely fine layer of protoplasm, which is easily detected in well fixed preparations.

The axis cylinder never loses this protoplasmic covering and, in my opinion, *both together enter the muscle fibre.*

It is very important, I think, to establish in this respect some definite point of view. This would save the investigators many an error. I personally strongly believe that the granular substance covering the ramifications of the nerve ending belongs really to this protoplasmic layer and not to the substance of the muscle fibre and that it must not be confounded with the sarcoplasm, as is done by some authors. As a distinguishing characteristic, I would emphasise that its capability of staining vitally with methylene blue as well as of reducing gold chloride is much stronger than is that of the sarcoplasm of the muscle fibre.

If this is so, it must be admitted that Kühne was quite right in distinguishing between two parts in terminal ramifications, viz. (*a*) threads of the axis cylinder (Axialbaum) and (*b*) a "stroma" substance, the nature of which Kühne does not define.

Kühne's observations were confirmed by Feist in a most definite form. He worked according to the method of vital staining with methylene blue and he established, in agreement with Kühne, that in terminal ramifications of motor endings it is possible to distinguish quite clearly a central, smoothly contoured thread intensely stained by methylene blue, and a less coloured peripheral mass (stroma, Kühne). When the preparation was fixed with picro-carmine, the peripheral part lost its blue colour and acquired a yellow shade, while the central thread preserved its former tint.

Retzius also says that such a differentiation was observed by him many times during his investigations, but he ignored these observations, taking them for an artificial result of treating the preparations with ammonium picrate.

A. Dogiel describes similar relations in reptiles, offering for them, however, no explanation.

Huber and de Witt, who also worked with methylene blue, affirm, that if the nerve terminations are examined immediately after being stained, no structure can be detected in the terminal ramifications. All branches of the axis cylinder are of an even blue tint. However, if in some 10–15 minutes the preparation is again exposed to the air, i.e. if the cover glass is taken off, it is possible to see, on examining it again, a central thread in many terminal branches. In preparations fixed with the ammonium molybdate, it is also sometimes possible to observe the presence of a central thread, but more often the axis cylinder ramifications appear to be finely granular. Huber and de Witt suppose that the differentiation which they observed and described depends upon the time which elapsed between the staining and the fixing of the preparation and upon the fixative used.

Personally, I can express quite a definite opinion upon the subject from my own observations and in an affirmative sense. The investigation of nerve staining according to the method of vital staining with methylene blue leaves, in my opinion, no doubt as to the fact that the hypolemmal terminal ramification of the nerve ending in the muscle fibre consists of a thin thread (perhaps a bunch of fibrillae) situated in the central part, always smoothly outlined and capable of being intensely stained with methylene blue. This portion is, in the strictest sense of the word, a continuation of the axis cylinder of the nerve fibre. Along the periphery of this central thread runs a thin layer of plasm, which is also stainable with methylene blue so that the terminal ramifications assume the appearance of the seemingly thickened continuations of the axis cylinder, but this peripheral portion of the terminal branches easily loses its staining and on being fixed with ammonium picrate becomes granular, the result being that the structure of the nerve ending stands out with complete prominence. The peripheral part of the nerve ramification consists of a substance capable of great expansion, and under some conditions of coagulation, and therefore varies in appearance under the microscope, being sometimes homogeneous, sometimes granular, the latter condition appearing always in fixed preparations, and at the same time the terminal ramifications acquire characteristic varicosities owing to the change in the peripheral layer. The central thread invariably remains thin and smooth. The nuclei situated along the terminal threads (the so-called "noyaux de l'arborisation," Ranvier) belong to the peripheral (plasmic) part of these latter.

Let us now pass to the distribution of the terminal ramifications under the sarcolemma of the muscle fibre, i.e. to the external form of the nerve endings. They are very characteristic in the frog. In this respect, the muscles

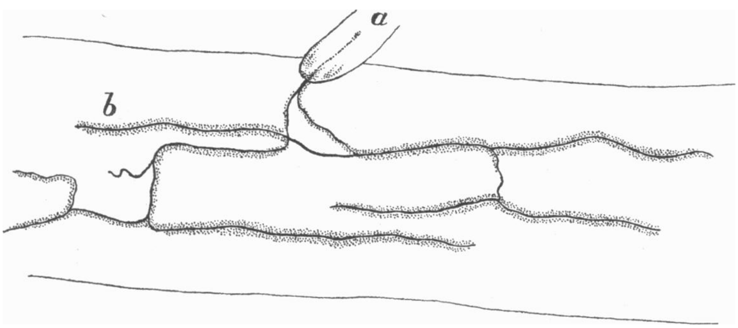

Fig. 1. Nerve ending in muscle fibre of frog; Methylene blue; *a.* medullated nerve fibre; *b.* ramifications of axis cylinders.

of Amphibia in general present a peculiar exception to all the rest of the Vertebrates. It has long since been established, that the axis cylinder of the nerve fibre immediately under the sarcolemma divides dichotomously, sometimes once, but mostly several times and that the terminal threads place themselves along the length of the muscle fibre. There are cases where the terminal threads are comparatively short, but more often they have the appearance of long branches, so that the nerve ending, which occupies a considerable portion of the muscle fibre, acquires its characteristic appearance, in marked contrast to the round or ovalish plates in other classes of Vertebrates.

Fig. 1 shows a typical form of the terminations in question. The axis cylinder of the nerve fibre, approaching the muscle fibre, divides into two branches. Then each branch in its turn divides 2–3 times. Each of the branches after the first division, however, forms already quite an independent little tree, the branches of which never anastomose with each other nor with the branches of the other tree. In this connection, I need only point out that all the observations of anastomoses between the branches of terminal ramifications are thought, by the authors themselves, to be more or less doubtful and are in evident need of confirmation. I myself have never seen any such anastomoses. The drawing given is quite accurate. All the branches are

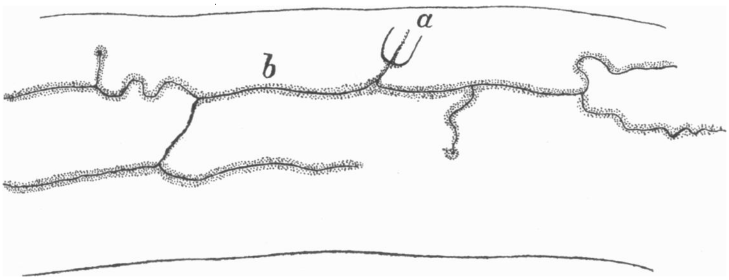

Fig. 2. Nerve ending in muscle fibre of frog; Methylene blue; *a.* medullated nerve fibre; *b.* ramifications of axis-cylinders.

indicated, including their distribution, and actual form. There are nowhere any interruptions or varicosities. I consider that also in life the terminal threads of the nerve endings have this or a similar appearance. The varicose shape of the terminal threads, so characteristic of all kinds of nerve endings, is in my opinion an artificial product, which appears as the first sign of the approaching post mortem changes or as the result of the methods of histological investigation generally employed.

Figs. 2 and 3 show the same kind of hypolemmal terminations, with the slight difference that some terminal threads give out short sprouts, ending with leaf-like enlargements (fig. 2). Sometimes these sprouts divide and then,

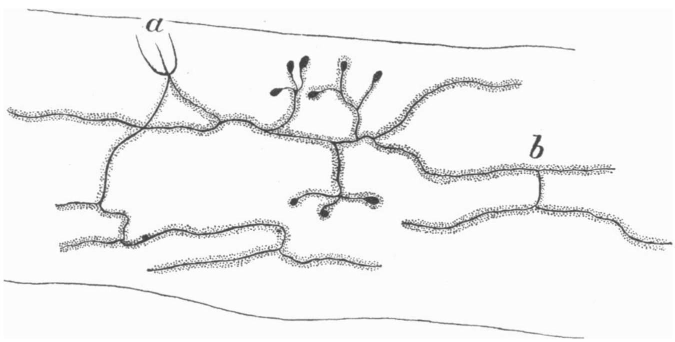

Fig. 3. Nerve ending in muscle fibre of frog; Methylene blue; *a.* medullated nerve fibre; *b.* ramifications of axis cylinders.

together with the leaf-like enlargements, they have the appearance of little bushes (fig. 3).

The ramifications of the terminal threads in question (*das Geweih* of German authors), preserving their character of ramifying threads stretched along the muscle fibre, present a variety of small deviations the forms of which are well known and do not require detailed description. But when Huber and de Witt say that generally "a further description seems superfluous," this should be taken "cum grano salis." As regards all questions concerning nerve terminations in muscles, even cardinal questions, we are far from possessing the precise data which would give us the right to finish with them once and for ever. Therefore I venture to draw the reader's attention to a few of these varieties.

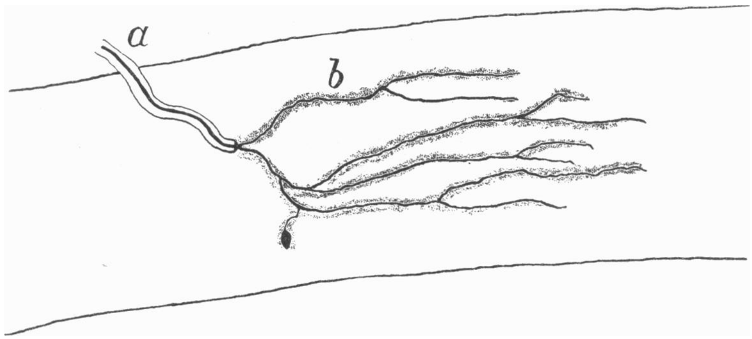

Fig. 4. Nerve ending in muscle fibre of frog; Methylene blue; *a.* medullated nerve fibre; *b.* ramifications of axis cylinders.

In one type, represented in figs. 4 and 5, the hypolemmal ramifications of the axis cylinder still present their usual character of long dichotomously dividing threads, but these latter do not place themselves along the extent of the muscle fibre, but, on the contrary, run across their length, and this difference is striking. We see it in figs. 7–8.

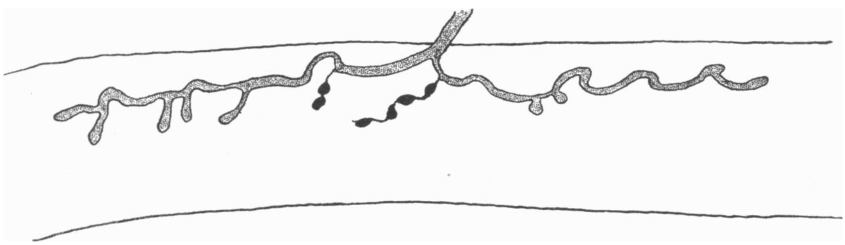

Fig. 5. Peculiar form of nerve ending in muscle fibre of frog; Methylene blue vital staining.

I should like to point out another very characteristic form which is very frequently encountered when the nerve termination is situated at the end of the muscle fibre. Usually, from the point of entrance of the nerve fibre into the muscle fibre, the terminal ramifications run along the extent of the muscle fibre both ways, as seen in figs. 1–3. At the ends of the muscle fibre it is possible to find, however, forms in which all the ramifications of the terminal threads direct themselves from the point of entrance of the nerve fibre towards the one side (fig. 4) and, if these ramifications are sufficiently numerous, they occupy the entire periphery of the muscle fibre. In this case the impression is that the end of the muscle fibre lies as it were in a basket-like plexus of nerve threads. Finally, I also met with forms which are difficult to place under any of the above mentioned groups. In fig. 5, we see how, from the point of entrance of the nerve fibre, thick branches run along the muscle fibre, from which similar sprouts arise, very short, sometimes taking the shape of hooks. Such endings have also been observed by other authors, cf. Kühne, fig. 70*e*. It is difficult to decide whether this is a special form of ending or only an anomaly or some phase of physiological state.

In concluding the description of the hypolemmal nerve endings, I think it necessary to point out, that I have only described those that are numerous enough to be regarded as permanent forms. The forms that are rare, or found singly, I omit, as these are either anomalous or may owe their aberrant character to the methods employed.

Some authors (A. Dogiel) touch on the question of whether each fibre receives only one motor nerve termination or several and whether it is supplied from only one *bunch* of motor nerves or from different ones. In this respect A. Dogiel expresses himself more definitely than others. He holds that the muscle fibre may be supplied with one, two or three terminal nerve apparatuses and in cases where the muscle fibre has only the one nerve apparatus this latter, he points out, is often of a compound kind, i.e. it consists of several ramifying terminal branches. In cases where the muscle fibre receives several (2–3) terminal nerve apparatuses one of them is usually compound, while the others appear in a simpler form.

The above description, however, is not quite adequate. Under the name of the terminal nerve apparatus must be included the whole of the axis cylinder ramifications which enter into the muscle fibre, for example the nerve fibre, approaching the place of its termination, usually divides once or twice, as mentioned above, and enters into the muscle fibre with two or four axis cylinders, which ramify under its sarcolemma, but in doing so they form one terminal nerve motor apparatus. There are cases where the medullated nerve fibre runs for some distance along the muscle fibre and gives off to it fine branches arising from the nodes of Ranvier; then eventually its axis cylinder enters in the same way as the branches into the muscle fibre (fig. 6). As a result we have a strongly developed terminal apparatus, but still only one and not several. A. Dogiel himself affirms, that in the case where the muscle fibre is supplied with several terminal nerve apparatuses *only one nerve fibre, which divides, as shown above, into* 2–3 *branches*, takes part in their formation.

I have met many times in the frog strongly developed terminal apparatuses. One of them is shown in fig. 7. The nerve fibre before termination divides into two branches, both of which, having lost their medullary sheath, enter into the muscle fibre and form, under its sarcolemma, extensive ramifications. These occupy the whole extent of the muscle fibre, but they still form one terminal apparatus. It seems to me that it would be an advantage if this point of view were accepted and if it were thus established that *every muscle fibre is supplied with only one terminal apparatus.*

This conclusion is not in conformity with the observations made by A. Dogiel, according to which the muscle fibre may receive its nerve apparatuses not from one nerve fibre only, but from various fibres approaching the muscle fibre from different sides. Such observations, however, are very doubtful as his

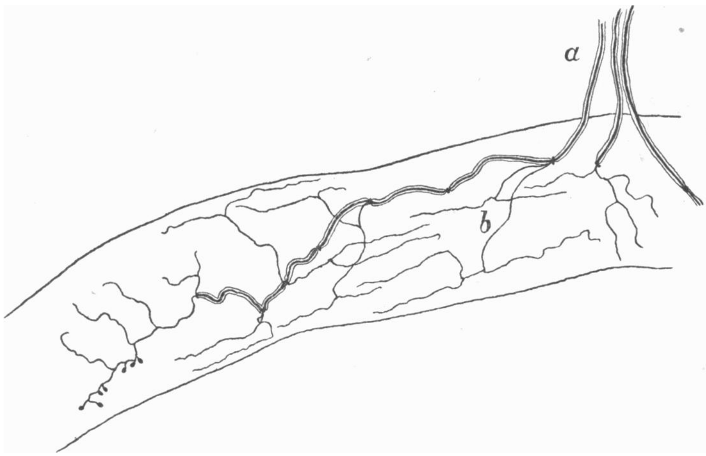

Fig. 6. Nerve ending in muscle fibre of frog; Methylene blue; *a.* nerve fibres; *b.* ramifications of axis cylinders.

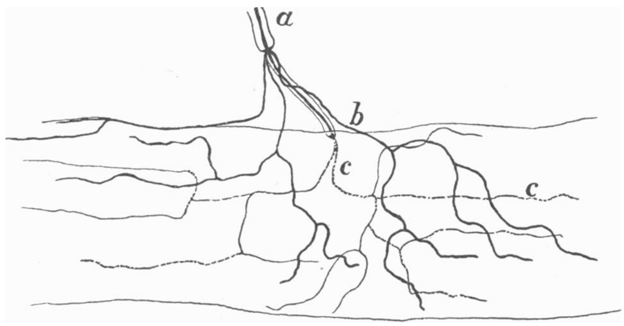

Fig. 7. Nerve ending in muscle fibre of frog; *a.* medullated nerve fibre; *b.*, *c.* ramifications of axis cylinders.

fig. 7 is not convincing. In that figure, both nerve fibres approaching the muscle fibre are broken and it is difficult to judge whether they are independent fibres or whether they are branches of a single fibre artificially separated at the moment of preparation. I have an analogous preparation. It is shown in fig. 8. We see two muscle fibres *A* and *B*, supplied by a medullated fibre *a*, which divides into two branches. One, thinner, terminates in the muscle fibre *A*, in the terminal ramifications of its axis cylinder. The thicker branch passes towards the muscle fibre *B*. Before entering the muscle fibre it, in its turn, divides into two branches, which terminate in fibre *B*, but one of these gives off a very thin branch *C*, which terminates in the muscle fibre *A*. If in our preparation we had encountered only one muscle fibre *A*, we should have concluded that it received two terminal nerve apparatuses from different nerve fibres, approaching from opposite sides, a conclusion which would have been incorrect.

This concludes my observations concerning the endings of the medullated nerve fibres of a motor character and I shall pass on to the description of sensory nerve terminations, but first I think it may be interesting to quote some references from histological literature.

Up to 1898 the question of nerve terminations in muscles, in spite of a colossal number of investigations, was in a distinctly unsatisfactory state.

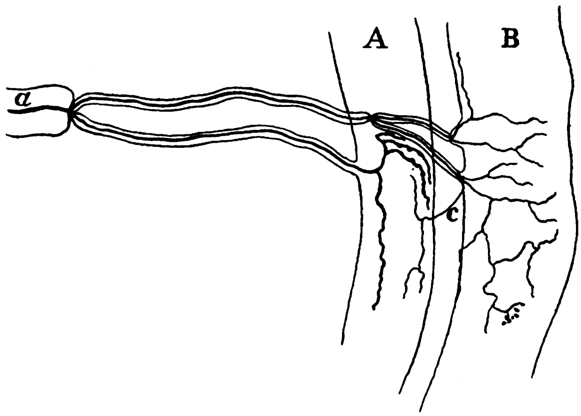

Fig. 8. Two nerve endings in muscle fibres of frog; gold chloride; *a.* medullated nerve fibre; *A.* and *B.* muscle fibres; *c.* additional nerve branch to muscle *A.*

A series of prominent investigators had described a great number of nerve terminations, very often quite precisely, but had given them such contradictory interpretations, that the whole question was left in a state of confusion. Such prominent observers as Kühne, Retzius, and A. Dogiel, accepted all the forms of nerve terminations in muscles, which they described, as being *motor* (hypolemmal) and only those of them that would not fit the established conception were distinguished by the name of "*atypical nerve endings.*"

Several of such atypical endings were described by Retzius in his classical work *Biologische Untersuchungen.* These forms were found by Retzius in the eye muscles of the rabbit and were regarded by him as motor terminations.

Retzius worked with methylene blue and his splendid drawings leave no doubt whatever of the fact that he made a very valuable observation and described the really original forms of nerve terminations.

In 1898, Retzius' observations were confirmed by Huber, but not in their entirety. Careful investigation of the nerve terminations in question brought Huber to the conviction that he was not examining the "*atypical forms*" *of* *motor terminations, but the normal forms of sensory nerve terminations.* Huber, besides, established the fact that these terminations lie outside the sarcolemma (epilemmally).

Huber's work, in my opinion, was of immense importance and established a new line of advance for the study of this difficult question, for we find that very shortly after Huber's work there followed a series of investigations which presented the question of nerve endings from a totally different point of view and opened out a region of sensory nerve terminations, which had been for long completely ignored. Such are the works of Crevatin, Ceccherelli, A. Dogiel, and others. I hope to return to these works later on when I investigate the nerve endings in the muscles of the Mammalia. Here I shall endeavour to describe the sensory nerve terminations in the muscles of the frog and to establish that even if the motor terminations in the frog's muscles stand somewhat apart from those of other Vertebrates, the sensory nerve terminations essentially resemble those in all the higher Vertebrates.

TERMINATIONS OF THE SENSORY NERVE FIBRES

In the frog we find the sensory nerve endings connected with medullated nerve fibres, as well as with non-medullated. It is clear, of course, that some of the non-medullated fibres are branches of medullated fibres. This is often the case, but it would be very difficult to accept it as a rule for all cases. The non-medullated fibres often run quite separately from the medullated and are distributed in places where there are no medullated fibres at all. Besides, the non-medullated fibres often form considerable plexuses, in which the medullated fibres usually do not participate. It is, however, hardly possible to affirm at the present time that the non-medullated fibres form an entirely independent system of fibres. Here I refer only to those sensory fibres, which terminate either upon the surface of the muscle fibres or in the interstitial connective tissue, and not to the vaso-motor nerve fibres.

The medullated nerve fibres of the sensory nerves run in bundles, always independently of the motor fibres, but, like the latter, they cross the bundles of muscle fibres in a transverse direction and gradually give off short, often ramifying, non-medullated threads at the nodes of Ranvier. These threads terminate with leaf-like expansions. In rare cases the terminal threads give

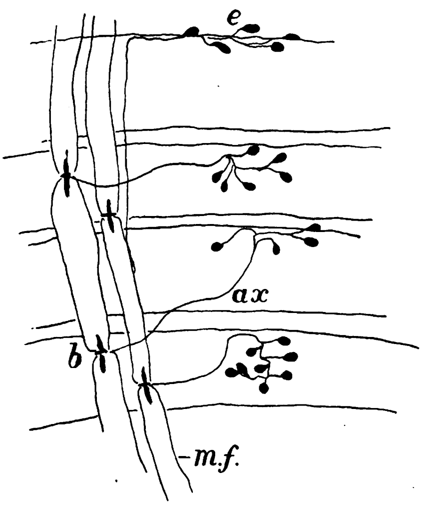

Fig. 9. Sensory nerve endings in muscle fibres of frog; Methylene blue; *ax.* axis cylinder; *m.f.* medullated nerve fibres; *b.* node of Ranvier; *e.* nerve endings.

off only 2–3 little branches. In the majority of cases the terminal thread splits into several branches each of which divides again. In such a case there would be quite a number of the leaf-like expansions. A nerve termination, whether large or small, always places itself on the surface of the muscle fibre, appearing in the shape of a plate, if examined from the side (in profile), but its real composition from threads with their leaf-like expansions always shows very clearly when it is examined from the surface (fig. 9). I should also point out that I possess preparations which indicate that the non-medullated threads given off from the medullated nerve fibre may be of a considerable

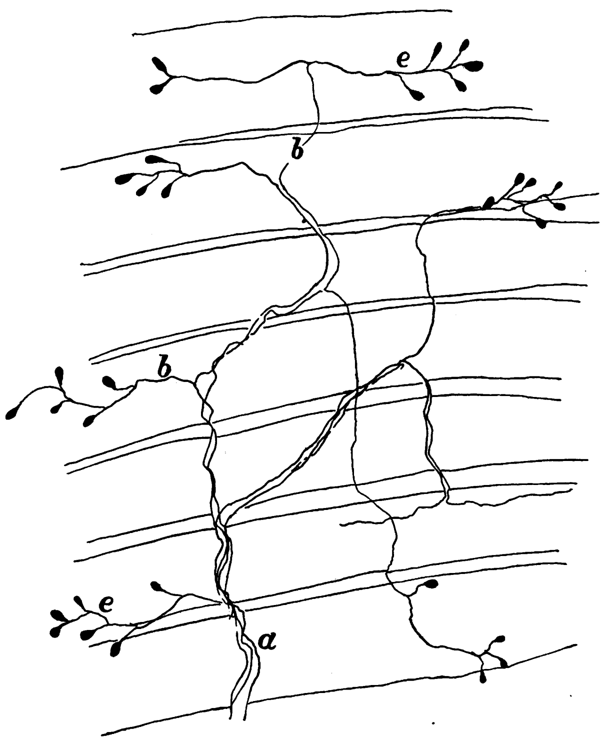

Fig. 10. Plexus of non-medullated nerve fibres in muscle of frog; Methylene blue; *a.* bundle of non-medullated nerve fibres; *b.* axis cylinders; *e.* nerve endings.

length. These terminate in the same way as the short threads, i.e. in a bush of ramifying threads with leaf-like expansions at their ends.

Fig. 10 shows the extensive ramifications of the non-medullated nerve fibres. These fibres may have sprung from medullated fibres, but in the preparation figured they have no connection with these latter. The principal branches also are evidently situated in a position more or less at right angles to the length of the muscle fibres, but fine branches and separate fibres run in the interstitial tissue along the muscle fibres for a considerable extent, as is seen in figs. 11–14.

Usually the bundles of non-medullated fibres, situated between the muscle fibres, preserve for the whole of their length a straight direction and curve only very slightly, but it often happens that they wrap over the muscle fibre and then they present a peculiar appearance, especially if the muscle is in a state of contraction (figs. 12, 13).

Here I must mention that the fibres in question were observed by Retzius in the eye muscles of the frog, but he took them to be motor fibres.

As to the terminations of the non-medullated nerve fibres, these are in principle everywhere similar and remind one of the already described terminations of the sensory fibres, derived from medullated fibres. The terminal

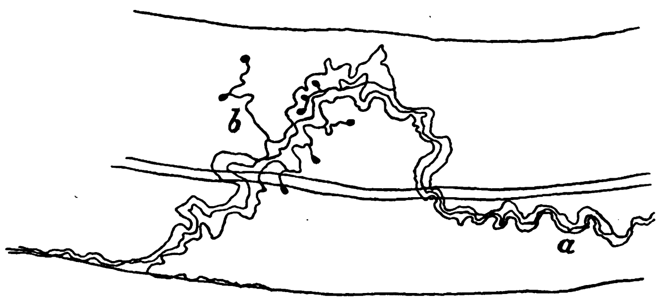

Fig. 11. *a.* Bundle of non-medullated nerve fibres in the interstitial tissue between the muscle fibres of frog; *b.* axis cylinders and their endings; Methylene blue.

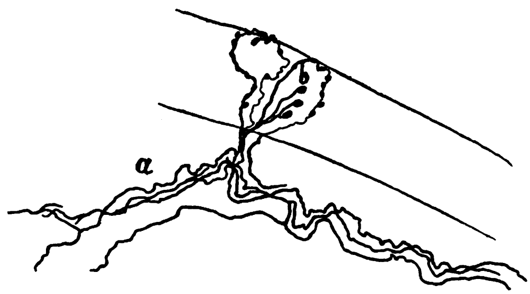

Fig. 12. Non-medullated nerve fibres in muscle of frog; *a.* bundle of non-medullated fibres; *b.* axis cylinders with their endings.

threads arise from the nerve bunch along the whole extent of the nerve fibre in the form of side sprouts, which terminate with leaf-like expansions. If the terminal threads have several ramifications, each one of the latter terminates with a leaf-like expansion and then the nerve termination acquires the appearance of a more or less considerable bush, but if it places itself upon the surface of the sarcolemma of the muscle fibre it acquires the shape of a plate. In individual cases, however, it is possible to come across a variety of forms in the described terminations.

In fig. 11 we see a characteristic bundle of non-medullated fibres, from which single short branches arise and terminate in leaf-like expansions. This is the simplest form of termination. The leaf-like expansions may touch the muscle fibre.

We find almost the same thing in fig. 12. The only difference lies in the

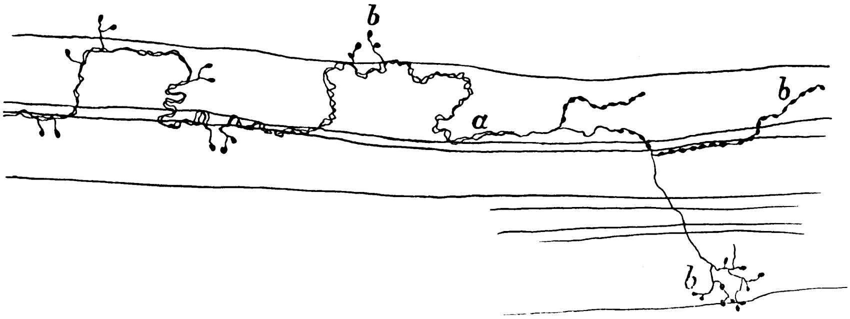

Fig. 13. Non-medullated nerve fibres in muscle of frog; *a.* nerve bundle; *b.* nerve endings; Methylene blue.

fact that here the nerve fibres wrap round the muscle fibre in the form of a fine bundle.

In fig. 13, the bundle of non-medullated fibres encircles the muscle fibre. From its fibres, the lateral terminal branches (*a*) arise, they divide dichotomously, and terminate in leaf-like expansions (*b*).

Fig. 14 shows a peculiar form. Along the muscle fibre a bundle of non-medullated nerve fibres is situated from which arise the side threads in a transverse direction, slightly ramifying. Each branch terminates in a leaf-like expansion with small terminal bushes. This form of termination was observed by Huber and Crevatin in the muscles of the eye in Mammalia. Fig. 14 very nearly approaches Crevatin's drawing.

I have taken for description only a few examples of these terminations. I do not attach any great importance to the forms described as such, as the principle in all these cases is the same.

Here I shall venture to leave the subject and to refer to the supposed special forms of nerve terminations, described for the first time by Giacomini under the name of nerve plexuses or "*terminaisons en réticelles.*" Unfortunately I am unable to refer to the papers of Giacomini and am only acquainted with them from the summary of Renaut et Regaut in the *Revue Générale d'Histologie* which seems to me to be excellent. The terminations "en réticelles" represent, according to Giacomini, the plexuses of thin non-medullated fibres round single muscle fibres or round small

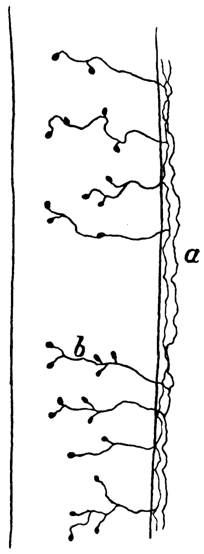

Fig. 14. Non-medullated nerve fibres in muscle of frog; *a.* nerve bundle; *b.* axis cylinders and their endings.

muscular groups. In the *Revue Générale d'Histologie*, Crevatin's fig. 30 is reproduced, evidently corresponding to the description of these terminations by Giacomini. From the thin and varicose threads arise short side branches, which terminate in free expansions (par des extrémités libres renflées). It seems to me that the terminations "en réticelles," which Giacomini described quite correctly as sensory terminations, were observed long before his investigations, but were incorrectly interpreted. So we find them illustrated by Retzius (1892) and even by Bremer (1882). Both authors observed them in the adult frog. The terminations of Giacomini (en réticelles), I think, correspond altogether to the description of the sensory terminations in the muscles of the eye in Mammalia (A. Dogiel, Crevatin) and to my present description of the sensory terminations in the muscles of the frog (figs. 11, 14). But I should not like to call them "terminaisons en réticelles." The fact is, that the non-medullated bundles, which give origin to this kind of termination, sometimes have an external likeness to plexuses. They sometimes encircle the muscle fibre with several rings, but they do not form any real plexuses.

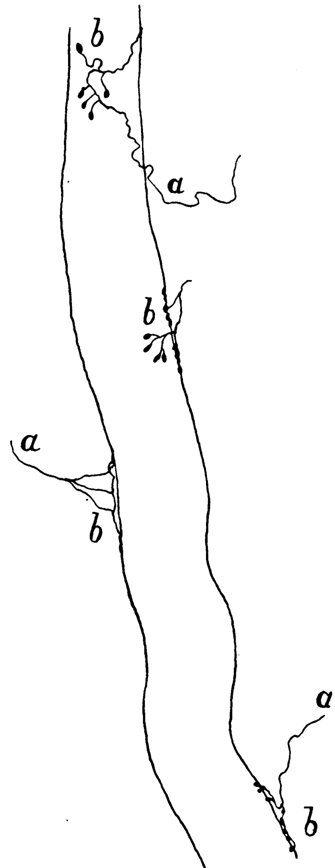

Fig. 15. *a.* non-medullated nerve fibres; *b.* axis cylinders with their endings; Methylene blue; frog.

It has been established by many authors that the muscle fibres are supplied with several sensory terminations. This is easily seen by observing the nerve fibres of the fine nerve bundles, which run along the extent of the muscle fibre and from time to time give off to it their terminal threads ending, as we saw above, in enlarged terminations or small bushes. I once succeeded in observing a muscle fibre which was supplied along a comparatively short distance with four plates of the typical form of sensory nerve terminations and, as I show in fig. 15, the terminations belonged not to one but to various nerve fibres. All the forms of sensory nerve terminations described by myself are, in my opinion, connected with muscle fibres. As I described above they all lie on the sarcolemma of the muscle fibre (epilemmally) and have neither granular lining nor nuclei. As to the structure of the said terminations, an important reservation is necessary. It is generally stated that the terminal thread whether short or long coming off from the fibre at the node of Ranvier erminates in an expansion. This is observed through the microscope under comparatively low powers. In preparations stained by the method of vital staining with methylene blue such terminal threads appear to be thin, straight, and more or less intensively coloured blue. The idea of former authors was that they represent naked axis cylinders, but if they are examined under a sufficiently high power ($\frac{1}{12}$ oil-imm.), it is not difficult to become convinced that the structure of the nerve endings is more complicated and that the terminal thread is covered by an extremely thin layer of a plasmatic substance unstained by methylene blue (in fixed preparations).

I do not think that all the sensory nerve terminations are immediately connected with the muscle fibres. Some of them probably terminate in the interstitial connective tissue. It is however very difficult to make a definite statement, perhaps for the reason that the form of termination of the terminal thread is most likely the same, i.e. the terminal threads terminate either in separate leaf-like expansions or in small bundles. In any case, I have not observed here those characteristic relations which I have described in the muscles of snakes (*Journ. of Anat.* LVIII.). Besides the non-medullated fibres, which I have described, and which I ascribe, probably in accordance with other authors, to the cerebrospinal nervous system, it is not difficult to observe in the muscles of the frog extensive plexuses of thin fibres connected with the blood vessels of all sizes, not excepting the capillaries. At the places where the nerve staining with methylene blue was sufficiently intense, relations are observed, which leave almost no doubt that these plexuses participate in the innervation of the muscle fibres. We know that this kind of nerve fibre belongs to the sympathetic system and it would be most desirable to throw some light on their relations to the muscle fibres from the morphological point of view, but this is not easy to effect. The fact is that the sympathetic nerves may not have the definite apparatuses in which the nerves of the cerebrospinal nervous system terminate, but that they may terminate in peculiar plexuses similar to those described by Perroncito and which personally I have sometimes seen in the muscles of the frog, although not in such a definite form.

SUMMARY

1. In respect of their nerve terminations, frogs form no exception to the rule, and in their muscles the same groups of nerve fibres are distributed as in the other vertebrates, i.e. the medullated and non-medullated fibres of the cerebrospinal nervous system and sympathetic fibres.

2. The motor nerve terminations lie under the sarcolemma (hypolemmally), the sensory on the outside (epilemmally). The old dispute about where the nerve terminations of the muscle fibres terminate is thus solved in a sufficiently satisfactory way.

3. The nerve threads entering the muscle fibre do not simply represent the so-called naked axis cylinders of the nerve fibre but are enclosed in a layer of plasmatic substance, which forms the peripheral part of the terminal thread. This plasmatic part of the terminal thread should not be confused with the sarcoplasma of the muscle fibre. The significance of the plasmatic part

is perhaps analogous to that of the granular substance (sole) of the motor nerves in Lizards, Birds, and Mammalia.

4. The distribution of the terminal threads of the motor nerve in the muscle fibre of the frog is very characteristic. The terminal threads have the appearance of long fibres, usually dichotomously branched, running chiefly along the length of the muscle fibre, although terminations are encountered in which the terminal threads take other directions.

5. Each muscle fibre receives only one more or less complex motor nerve termination. One nerve fibre only participates in its formation. This latter, on dividing, may certainly supply with nerve terminations a considerable group of muscle fibres.

6. The nerve terminations which were described by Kühne, Retzius and A. Dogiel as "atypical" forms of motor terminations should be ascribed to the sensory nerve endings, as was first pointed out by Huber.

7. The sensory nerve terminations of the spinal nerves in the frog fully correspond to similar terminations in Mammalia. All forms of such terminations in these latter described by authors are very clearly observable in the frog.

8. In the frog *medullated* as well as *non-medullated* fibres terminate in sensory nerve endings.

9. In its essence, every sensory termination presents a thin terminal thread terminating with a leaf-like expansion. If the terminal thread is ramifying or gives off side sprouts, the termination becomes more complicated and appears in the shape of a nerve apparatus more often having the appearance of a plate lying with its expansions upon the sarcolemma. The sensory nerve terminations, whether they belong to medullated or non-medullated nerve fibres, have all essentially the same constitution.

10. The muscle fibre may be supplied with several sensory nerve terminations.

11. It is very possible that the structure of the sensory nerve apparatuses is more complicated than is described by authors at the present time. There is reason to conclude that the terminal threads of these terminations, as well as those of the motor, are enveloped in plasmatic substance.

12. It must be held that part of the non-medullated nerve fibres terminate in the interstitial connective tissue.

In a subsequent paper, I hope to deal with the sympathetic nerve endings in the muscles of the frog.

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I wish also to avail myself of this opportunity to express my heartfelt thanks to the Professors of University College—Prof. G. Elliot-Smith and Prof. J. P. Hill—for their invariably friendly consideration for myself and my work.

BIBLIOGRAPHY

(1) ARNSTEIN, C. (1887). "Die Methylenblaufärbung als histologische Methode." *Anat. Anzeiger,* N. 5, n. 7.

(2) BREMER, L. (1882). "Ueber die Endigungen d. markhaltigen u. marklosen Nerven in quergestreiften Muskel." *Arch. f. mikroskop. Anatomie,* Bd. XXI.

(3) CUCCATI, G. (1888). "Delle terminazioni nervee nei muscoli abdominali della rana temporaria e della rana esculenta." *Monthly internat. Journ.* vol. V.

(4) —— (1889). "Intorno al modo onde i nervi si distribuiscono e terminano nei muscoli abdominali del triton cristatus." *Ibidem,* vol. VI.

(5) DOGIEL, A. (1890). "Methylenblautinctur der motor. Nervendigungen in d. Muskeln d. Amphibien u. Reptilien." *Arch. f. mikr. Anatomie,* Bd. XXXV.

(6) —— (1901). "Die Nervendigungen im Bauchfell, in den Sehnen, d. Muskelspindeln, etc." *Ibidem,* Bd. LIX.

(7) —— (1906). "Die Endigungen d. sensiblen Nerven in d. Augenmuskeln u. deren Sehnen beim Menschen u. den Säugethieren." *Ibidem,* Bd. LXVIII.

(8) FEIST, B. (1890). "Beiträge zur Kenntniss d. vitalen Methylenblaufärbung des Nervengewebes." *Arch. f. Anatomie u. Entwicklungsgeschichte Anatom.* LXVI.

(9) HUBER, CARL (1899). "A note on sensory nerve endings in the extrinsic eye muscle of the rabbit, 'atypical motor endings of Retzius.'" *Anat. Anz.* N. 15.

(10) HUBER, CARL and DE WITT (1898). "A contribution on the motor nerve-endings and on the nerve-endings in the muscle spindles." *The Journal of Comparative Neurology,* vol. VII.

(11) KÜHNE, W. (1887). "Neue Untersuchungen über motorische Nervendigung." *Zeitschr. für Biologie,* Bd. V (Neue Folge).

(12) PERRONCITO, A. (1902). *Studi Ulteriori sulla terminazione dei nervi nei muscoli a fibre striate.* Pavia.

(13) RANVIER, L. (1875–82). *Traité technique d'histologie.*

(14) REGAUT, CL. et FAVRE, M. (1904). *Revue générale d'histologie,* Tome I.

(15) RETZIUS, G. (1892). *Biologische Untersuchungen,* Neue Folge III.