THESIS

for

DEGREE OF M.D.

on

"The Nerve Endings in the Panniculus Carnosus of the Hedgehog with Special Reference to the Sympathetic Innervation of Striated Muscle.

by

HUGH SHAW DUNN GARVEN, B.Sc., M.B., Ch.B.

----oOo----

I. Introduction,

II. Review of Previous Histological Investigations.

1. On the structure of muscle fibres with special reference to the existence of two types of striated fibre.
2. On the constitution of the muscular nerves.
3. On motor, sensory, and accessory nerve endings in striated muscle. (exclusive of neuro-muscular spindles).
4. On neuro-muscular spindles.

III. Original Work. 53

1. On the Panniculus Carnosus muscle of the hedgehog.
    - (a) The muscle fibres.
    - (b) The constitution of the nerves supplying this muscle.
    - (c) The motor and accessory nerve endings.
    - (d) The neuro-muscular spindles.
2. Note on nerve endings in striated muscle of the frog.
3. Note on nerve endings in striated muscle of the lizard.
4. Note on nerve endings in extrinsic eye muscles of the rabbit.
5. Note on nerve endings in pectoral muscles of man.

IV. Discussion. 86

V. Conclusions.

VI. Description of Figures.

VII. References.

VIII. Plates.

Introduction.

The existence and function of the sympathetic innervation of striped muscle has been much discussed since Boeke's discovery of his accessory nerve fibres and endings. In this work on mammalian muscle, Boeke used the Bielshowsky silver method of staining nerve fibres and neuro-fibrils and demonstrated the presence of a medullated and non-medullated (sympathetic) nerve supply to the individual muscle fibres. This means a double innervation of the individual muscle fibres in the sense that the two nerve fibres are of entirely different origin. Upon this foundation has been built up the theory, that the cross-striped apparatus of the fibre is innervated by the somatic fibres and is responsible for muscular movements and contractile tonus, using Langelaan's distinctions, while the sarcoplasm of the fibre is innervated by the sympathetic and is responsible for plastic tonus.

This conception has recently been modified by Hunter, on the basis of Kulchitsky's histological work. Kulchitsky, using the gold chloride method of staining nerve fibres and nerve endings, has recently in reptilian muscle (Python) described two types of nerve endings, which never occur on the same muscle fibre. One of these is supplied by a medullated nerve fibre and the other by a non-medullated nerve fibre, but these two types he has never found on the same muscle fibre. Hunter suggests that one type of muscle fibre, innervated by somatic fibres, is/

is responsible for muscular movements and contractile tonus, while the other type of muscle fibre, innervated by the sympathetic, is responsible for plastic tonus. This means a double innervation of every muscle as a whole but not of each individual muscle fibre.

A third complication has arisen in the use of the term "double innervation," for while the traditional view has been that each muscle fibre receives one medullated nerve fibre, Agduhr, in recent investigations on limb muscles, has shown that each muscle fibre is segmentally innervated. This means that the individual muscle fibres of a muscle, innervated from more than one spinal root, has an end-plate from each root; that is, a double innervation in the sense that the individual muscle fibre is supplied by two or more somatic fibres.

This short summary of the present position with its evident contraditions emphasises the need for further detailed histological research on the neuro-muscular junction.

I regret that on the basis of my original observations I have to disagree with two of these observers, Professors Kulchitsky and Hunter, who have so suddenly been cut off in their scientific careers, and whose loss will be deeply felt in the solution of these problems.

At the outset, this work was undertaken to attempt to confirm the presence of Boeke's accessory fibres and end-nets by the classical gold chloride method. Since the publication of/

of Hunter's work various other questions have arisen in conjunction with this primary aim, and these will also be dealt with. The first question which presents itself is the choice of a muscle for histological investigation. Mammalian muscle is preferable to reptilian or amphibian muscle as the application of the findings must be made more especially to the function of mammalian muscle. In view of the fact that the sympathetic innervation is supposed by some to be of tonic function - a muscle showing marked tonus, especially plastic tonus, is also preferable. For these reasons the panniculus carnosus muscle, of the hedgehog - a sheet of striped muscle which lies just under the skin over its whole dorsal surface and curls the animal into a ball - was chosen. Pembrey (1903)(45) has shown that this muscle is in tonic contraction during the whole period of hibernation and it will, I presume, be maintained in its shortened condition by plastic tone.

Some reference will also be made to some investigations on frog, lizard, rabbit and human muscle, which show several conditions of innervation which are of interest in relation to the main thesis.

I propose to review some of the previous literature on this subject under the following four headings:-

1. On the structure of the muscle fibres, with special reference to the general existence throughout vertebrates of two types of striped muscle fibre.

2/

2. On the constitution of the motor nerves to muscle with special reference to the size of the nerve fibres and the existence of non-medullated fibres in the nerve before it enters the muscle.

3. On the nerve endings in muscle, motor, sensory, and accessory, with special reference to the existence of the accessory system. In reviewing this work I do not propose to cover the whole field of older investigations: it is sufficient to note that the great majority of these workers using the gold chloride method made no mention of this system of fine non-medullated fibres. However, in relation to the present investigations, those papers in which some mention is made of non-myelinated fibres found associated with muscle fibres, are important and will be dealt with in more detail. The later work where these accessory fibres, or fibres like them, are described, will also be dealt with in detail.

4. On the special sensory organs in muscle - the neuromuscular spindles.

PART II.

------oOo------

I. On the Structure of Muscle Fibres, with Special Reference to the General Existence throughout vertebrates of two Types of Striped Muscle Fibres.

The existence of two types of striped muscle fibres in the frog has been long known. An examination of fresh frog muscle in saline shows clearly that all the muscles of the frog are composed of clear, bright, broad fibres with very well marked cross striation and poorly marked longitudinal striation, and of dull granular thin fibres with poorly marked cross-striation, and well marked longitudinal striation. Ranvier (1887)(49) first drew attention to the marked difference in the muscles of the rabbit, the red muscles and the white muscles. Similar differences are found in the muscles of birds.

Schafer (1912)(54) noted that in other animals the distinction is not found as regards whole muscles although it may affect individual fibres of a muscle. He quoted Klein as showing that some fibres of the diaphragm in distinction to others have a very large number of nuclei and these are embedded in protoplasm.

Grützner (1884)(29) in his paper on the two types of muscle fibres, held that the thin granular fibres found in frog's muscle are analogous to the red fibres of the rabbit muscle and that the broad clear fibres of frog's muscle are represented in rabbit muscle by the white fibres. He also contended that all mammalian muscles are composed of the two types of individual fibres, red and white, as the individual frog muscles are composed of thin granular fibres and broad clear fibres, the only difference being that in the mammalian muscles there are larger amounts of haemoglobin.

Grützner used various methods of differentiating the two types, Acetic Acid, Osmic Acid, Picric Acid and Iodine. In his paper he gave diagrams of cross sections of muscles of the guinea pig; the two types of fibres were shown but differ from the frog in this, that the one type of fibre was not smaller than the other and it appears that the two types are not so clearly differentiated as in the frog, some fibres occupying an intermediate position. His most interesting statement is made in regard to human muscle. In this material he stated that the two types were differentiated from one another less by differing size and colour than by a dullness ("Trübe") in some of the fibres, when treated by his Acetic Acid method, where the muscle is dried, sectioned, and put in 2% Acetic Acid. He explained the failure of other workers to find this difference because, if any clearing agent is used in the process, no differentiation can be found and also because muscles taken from the post-mortem room hardly show it, even when treated by his drying method.

The/

The facts that in mammalian muscle there is very little difference in size in the individual fibres, that such a method as drying is necessary to show a difference in the dullness or brightness of the fibres, that the results with this method do not differentiate the two types of fibres into very clear groups, and that no difference can be found with ordinary methods, seem to force one to the conclusion that the two clearly differentiated types of fibres, which appear in the frog, do not exist in this type of muscle. All that can be said definitely is that there may be fibres which contain slightly more sarcoplasm than their fellows.

Grützner did not formulate a general law of the function of the two types of fibres, but suggested that the red fibres are related to tonic contraction, and the white fibres to rapid contraction. Since he held that every muscle had varying proportions of white and red fibres, and that no muscle was completely red or white, he found it very difficult to prove definitely that the muscles having a larger proportion of red fibres, showed a longer or more tonic type of contraction, or vice versa.

It is important to note that in Grützner's paper no mention is made of the muscle fibres in the neuro-muscular spindles. These spindle muscle fibres in mammalian muscle, at first considered to be groups of embryonic fibres, but now known to be fully developed fibres performing probably a proprioceptive function, show a marked difference from the mass of fibres in the/

the muscle. They are thin granular fibres with well marked longitudinal striation, and are enclosed in a laminated connective tissue sheath. In my opinion they correspond to the thin granular fibres of the frog muscle, some of which have nerve endings upon them showing that they are rudimentary neuro-muscular spindles and they only differ from the Weissman fibres in mammalian muscle, in that they are not collected in the complex neuro-muscular organ but are distributed throughout the body of the muscle between the broad clear fibres and are not walled off by a laminated sheath.

Cilimbaris,(1910)(20) in a paper on the muscle-spindles of the eye muscles, stated that in them he found great variations in the ordinary muscle fibres such as, he remarked, one could scarcely ever find in any other part of the mammalian striated musculature. Some fibres appeared clear, were poor in sarcoplasm, and had few nuclei, while others appeared dark, were rich in sarcoplasm, and had many nuclei. He noted quite definitely that all intermediate stages between these two types could be seen.

Ffrangcon Roberts (1916)(51) in a paper gave observations on the degeneration of muscle after nerve injury, especially the red and white muscles of the rabbit. He stated "In striped muscle, fibres of two kinds are found, the white and the red. .... In man each individual muscle is composed of a mixture of both kinds of fibres, while in some animals, notably the rabbit, different muscles are composed of one kind or the other, the/

the gastrocnemius for instance being purely white, and the soleus purely red." The red fibres, he said, had more sarcoplasm than the white fibres and responded with a sluggish contraction after a long latent period, while the white fibres responded briskly. He quoted no authority for his statement regarding human muscle nor did he advance any proof of it in his own observations.

Kahn (1924)(34) at the outset of his research on the chemical constitution of the clasping muscles of the frog at different seasons, made some histological observations which are of great interest from the point of view of the two types of muscle fibre. In Rana fusca he found that the M. flexor Carpi Radialis was composed in the autumn and early spring of many large bright cross-striped fibres with only a few thin sarcoplasm rich fibres with poorly marked cross striation. These differences he found on cross section to be shown very obviously after fixing in formal alcohol and staining with picro-fuchsin and haematoxylin. In the breeding season the same muscle was examined. On cross section the muscle appeared on first examination to show a larger number of fibres per unit area, that is, that the individual fibres were smaller in area. In the fresh preparation the preponderating number of fibres were definitely shown to be clear, large fibres as in the autumnal muscle and a close examination of the cross section showed that the number of small granular fibres was not increased. The appearance of a larger number of fibres per unit area was due to the fact that in the breeding season a number of fibrous/

fibrous filaments passing in from the sarcolemma of the large clear fibre to the central parts of the fibre gave the impression of incomplete longitudinal division of the large fibres. In the autumnal muscles the Cohnheim's areas were not well marked, but in the breeding season they were very well marked off, and in some cases the division between the groups of Cohnheim areas appeared to have the extensions of the sarcolemma mentioned above, passing in to the middle of the fibres.

These appearances Kahn thought were obviously dependent on the fact that in the breeding season there was a larger content of sarcoplasm in the large clear fibres, compared with these fibres in the autumn, for the sarcoplasm made the Cohnheim areas more obvious and the cross section of the sarcostyle more distinct. And most important from our point of view, he definitely stated that the richness of sarcoplasm was by no means so large in the breeding season that these fibres could be mistaken for the small granular fibres. The increased tonic activity of the muscle was therefore maintained by a change in the large clear fibres themselves and not by an increase in the number of small granular fibres.

Hunter (1925)(33) after reviewing the work of Ranvier and Grützner said that it remains to be decided whether or not all striated muscle is composed of two types of muscle fibre. To support his hypothesis that these two types of fibres are innervated from different sources and fulfil different functions he brought forward first of all evidence that the two types of muscle/

muscle fibres do exist in the leg muscles of the goat and the wing muscles of the domestic fowl. In referring to this work he did not make mention of Grützner's observations that clearing agents destroy any possibility of making the differentiation, and in both the investigations on goats and birds, Hunter relied mainly on the size of the fibres which Grützner had already shown not to be a feature of the differentiation in mammalian and avian muscle.

In goats Hunter and Royle (33) did not state whether any variation in the size of different muscle fibres was seen in the normal muscle, but relied upon degeneration experiments to bring out the difference. They removed the lumbar sympathetic chain from one side and after six months examined the muscle in cross section. They found a large number of small fibres in contrast to fibres of normal size. These they stated were too numerous to be the conical ends of fibres of normal size. The endomysium between the individual fibres was increased. This experiment, in my opinion, in no way proves the existence of the two types of fibre in the normal muscle.

In the bird, they stated definitely that remarkably little variation in the size of the individual fibres in the normal muscle was found and presumably no other difference was observed, else it would have been mentioned. In his degeneration experiments, the last three cervical roots, anterior and posterior, were cut without injury to the Rami Communicantes and the flexors of the forearm, examined in cross section after seventy days.

On /

On section many fibres in groups were found to be of normal size or larger, other fibres, also in groups, showed a 33% decrease in size, while other groups were found with both normal and shrunken fibres. The connective tissue was increased in amount. They quoted that Willard and Grau had found a progressive decrease in size up to 68% on nerve section. The inference therefore seems to be that the degeneration was not complete and may have affected some fibres more quickly than others. It is also worthy of note that the somatic denervation of the limb was not complete and if Agduhr's contention is correct, that muscle fibres are themselves innervated plurisegmentally, then some of the fibres may still have had some part of their somatic innervation. If however the somatic denervation and degeneration was complete in those fibres showing a decrease in size: then in them degeneration was not so complete as when motor nerve section (motor and sympathetic fibres) was performed. The intact sympathetic innervation may be held to be the cause of this less complete degeneration.

Hunter's conclusions, from his degeneration experiments, that somatic or sympathetic denervation affects some fibres and not others and that therefore there are two types of muscle fibres mixed in mammalian or avian muscles seems to me to require much better evidence than is afforded by these experiments.

It/

It seems reasonable to suppose that if the two types of muscle fibre exist together in the mass of mammalian or avian muscle, then the differentiation into the two types should be demonstrable in normal muscle and this Hunter and Royle have not been able to show.

Bottazzi (1923)(15) in a communication to the International Physiological Congress, 1923, said that in dogs after section of the roots of the phrenic, leaving the sympathetic fibres intact, if sufficient time had elapsed - the diaphragm was almost completely degenerated on the side of section. He made no mention of histological investigation but presumably no irregularity in the degeneration was noted.

----

2. On the Constitution of Motor Nerves to Muscle.

One of the great difficulties that presents itself while reviewing much of the earlier work on the innervation of striped muscle, lies in the fact that so very often, when non-medullated fibres are figured or described, they can only be followed and are described for a very short distance just proximal to the end-plate or termination. The question at once arises, are these non-medullated fibres simply branches of the medullated fibres proximal to this point or are they non-medullated throughout their whole course, and if so, are they to be found in the motor nerve before it enters the muscle?

Sherrington (1894)(55) made a very extensive study of the constitution of the muscular nerves and the function of the different fibres contained in these nerves. Some of his conclusions are of interest. One third or one half of the nerve fibres in a nerve to a muscle are sensory, and the smallest of the myelinated fibres were for the most part if not entirely also sensory. These nerves contained no myelinated fibres of sympathetic origin, but did contain a number of non-myelinated fibres of sympathetic origin. Some of these non-myelinated fibres were of larger diameter than the small myelinated fibres.

Ranson (1911)(48) has shown with the silver impregnation method that the spinal nerves and posterior roots contained a very large number of deeply stained fibres, which were non-myelinated and arose in part from the small ganglion cells of

the/

the spinal ganglion, and were afferent in function since they were too numerous to be entirely sympathetic.

Langley (1922)(40) in his paper on the constitution of nerve roots, muscular nerves, and cutaneous nerves, found with the osmic acid method very few non-medullated fibres in the nerve roots and suggested than Ranson's fibres were really fine medullated fibres. In comparing muscular and cutaneous nerves, a point which is of special interest regarding the sympathetic innervation of striated muscle, Langley found that the nerves to skeletal muscle contained very few non-medullated fibres whereas the cutaneous nerves showed many non-medullated fibres. He even went so far as to say that his results were not in favour of any considerable connection of non-medullated nerve fibres with striped muscle fibres. He also brought out the point that a large factor in determining the size of the nerve fibre was the nature of the tissue with which it was connected.

Sherrington (1894)(55) had already shown that most of the larger medullated fibres in a muscular nerve were sensory in function, ending in the neuro-muscular spindles.

It is only necessary to note at this point that it is very doubtful whether the osmic acid method would enable such thin nerve fibrils, as those described by Perroncito,(1902)(47) to show sufficient contrast to the connective tissue as would enable one to distinguish and ennumerate them.

----

3. On the Motor, Sensory, and Accessory Nerve Endings in Striped Muscle, exclusive of the Neuro-Muscular Spindle Endings.

Tschiriew (1879)(57) first differentiated two types of end-plates in Tropidonotus natrix, the usual Rouget plate with a well-marked granular sole-plate and a medullated nerve fibre forming the terminal arborisation, and the "terminaisons-en-grappes" as he named them. These shewed only a very finely granular sole-plate, or were without the granular substance altogether, were found on thinner muscle fibres with clear and well marked striation and usually a non-medullated nerve fibre formed the arborisations. He found all stages of transition types between the Rouget plates and the "terminaisons-en-grappes," and regarded the latter as developmental forms of the former.

Bremer (1882)(18) in his excellent paper on the endings of myelinated and non-myelinated nerve fibres in striped muscles, worked upon the muscle of frogs and lizards mainly. In the frog, he differentiated three types of nerve fibre, (I) thick medullated fibres - medullated fibres of the first order, (II) thin medullated fibres - one quarter or one fifth of the diameter of those of the first order - medullated fibres of second order, and (III) definitely non-medullated fibres of the third order. Bremer thought that the third order was derived from the second, although, he remarked, in some cases, that in the very long course through which a non-

non-myelinated fibre could be traced, it showed no connection with medullated fibres of the second order. The thickly medullated fibres formed typical end-bushes of Kuhne. The thin medullated fibres formed endings of a different type, the 'end-dolden' or umbel forms, which were, Bremer thought, the same as the terminaisons-en-grappes of Tschiriew. The non-myelinated fibres formed plexuses in the muscle and ended in forms conforming in essentials to the 'dolde' type but smaller, as a rule.

In the M.Hyoglossus of the frog, he described several conditions which are of special interest. In the first place the distinction between fibres of the first and second orders had been almost completely lost, and often the fibres of both orders lose their myelin a long distance proximal to their endings. In this muscle, the typical end-bush of Kuhne was relatively infrequent and the 'umbel' form very frequent. Further he described cases in which a non-medullated fibre branched dichotomously and gave one branch to a muscle fibre where it ended in an umbel form, and the other branch to a blood vessel. More rarely he stated that this double innervation of muscle and blood vessel took place from a medullated fibre of the second order.

Again in the M. Lingualis he described small ganglion cells connected with the non-myelinated fibres or the myelinated fibres of the second order. The branches of the fibres arising from these ganglion cells passed either to the blood vessels or to the/

the muscle fibres. In this muscle also the characteristic form was the umbel. It was in this muscle that he found that, while the majority of the non-myelinated fibres could be traced to origins from the myelinated of the second order, some of these could be traced as free fibres throughout a long course. This point seems of interest in relation to later work. Another peculiarity he described in this muscle, is interesting in relation to Boeke's work; in some cases the non-myelinated fibre broke up into thin fibrils only to unite again to form a single fibre after a very short course. Instances of the medullated fibre of the second order giving branches to both blood vessels and muscle fibres were commoner here than in the M. Hyoglossus.

Several points are worthy of mention, in connection with Bremer's figures of the nerve endings in frog muscle. Fig. 6 shows a medullated fibre of the first order forming an end-dolde. Fig. 14 makes it clear than end-dolden forms and end-bushes can be found on the same muscle fibre. Figs. 6, 7, and 9 which show end-dolden forms and fig. 8 which is described as an intermediate form between the dolden and bush forms, show accompanying the myelinated fibres, which form the chief part of the end ramification, fine non-medullated fibres running inside the sheath of Henle. These thin fibres take some part in forming the end-apparatus, without, it may be, any direct anastomosis with the end ramifications of the medullated fibre. Some of his figures of the umbel form show a well marked granular sole, while others do not.

In/

In the lizard, Bremer worked upon the triceps and tongue muscles. In the triceps the same three types of nerve fibres were found. The medullated fibres of the first order ended in the well-known end-plates of Rouget. The fibres of the second order ended in umbel endings, - elongated plate-like endings. These fibres often lose their myelin proximal to the endings. Fig. 21 shows an umbel form, which is very like Kulchitsky's figures, but it shows a well-marked granular substance. The non-myelinated nerve fibres Bremer found often running in the same sheath as the fibres of the first order and they assisted in forming a common plate with the medullated arborisations. In the tongue the nerves were found, as in the frog, to be very difficult to classify. In the lizard muscle he stated definitely that one muscle fibre could have two end-plates. He also found that one muscle fibre could have two or more end-umbels, or one end-plate and one or more end-umbels.

In reviewing the work of Tschiriew on the terminaisons-en-grappes, which are the same as his umbel forms, Bremer stated that while Tschiriew's terminaisons-en-grappes were formed from medullated fibres which become non-myelinated a long distance proximal to the ending, his umbel forms were endings of the myelinated fibres of the second order. Some of his figures however do not altogether bear this out, and in this generalisation no mention is made of the intermediate types, between end-bush and end-umbel, which he described. From the physiological point of view, Bremer was very guarded in his suggestions, but he leaned/

leaned to the view that the fibres of the first order were probably motor and the fibres of second and third orders (non-myelinated) were probably sensory.

Sherrington (1894)(55) stated that these end-dolden forms of Bremer have been proved to be of motor function.

Retzius (1892)(50) also described atypical motor nerve endings in the extrinsic muscles of the eye. These endings were derived from myelinated fibres which proceeded for a long distance after losing their myelin, as non-myelinated fibres, or from thin non-myelinated fibres, which passed out from a myelinated fibre at a node of Ranvier. The endings are simple, disc-like and deeply staining and may be single, or several arranged in a linear series or in small clumps. More complicated forms were also found.

Huber and De Witt (1898)(32) using the Methylene blue method found numerous examples of the atypical endings of Retzius, formed from fine non-myelinated fibres branching off from the medullated fibres at the nodes of Ranvier. These thin fibres ended in end-discs - either singly or several together, forming a more complicated ending. A number of these endings could be found on one muscle fibre. No group of muscle nuclei or eminence of Doyère was found in relation to them. They believed that,unlike the motor endings, these end-discs lay outside the sarcolemma in the connective tissue between the muscle fibres. They found the medullated nerves forming these end/

end-discs to have very short internodal segments and a well-marked nucleated fibrous sheath. For these reasons they thought them sensory endings.

Huber (1899)(31) using the same method examined the extrinsic eye muscles confirmed the presence of Retzius' atypical motor endings but for the reasons above he thought them sensory. He found also that these sensory terminations stained much more easily with methylene blue than did the typical motor endings, probably because they lie outside and not inside the sarcolemma. He noted that while the motor terminations were found mostly in the middle third of the muscle, the sensory terminations lay mostly in the anterior third. He found no other sensory organs in the extrinsic muscles. The motor nerves were found to branch and rebranch although one small bundle supplies a relatively small area; single fibres could not be traced for any considerable length. The sensory fibres on the other hand could be traced running longitudinally in the muscle for considerable distances, showed very short internodal segments and after losing their myelin could be traced for a long distance.

Ruffini(1900)(53) at an Anatomical Congress at Pavia demonstrated the existence of a thin non-myelinated fibre which he called the ultra-terminal fibre, in the muscles of the thenar eminence of man. From one of the terminal ramifications of the motor plate a very fine fibril passed out, but he could not definitely state its destination.

Perroncito/

Perroncito (1901)(46) working on reptilian muscle confirmed the presence of an 'ultra-terminal fibre.' This thin non-myelinated fibre he found to pass out from one of the ramifications of a Rouget end-plate, or from the point of entrance of the medullated nerve fibre into a plate, or from the medullated fibre before it entered the plate. It forms a rudimentary plate either on a neighbouring fibre or on the end of a thin muscle fibre of the simple neuro-muscular spindle found in lizards. One case also he showed of a medullated fibre dividing into two medullated branches, one of which formed a Rouget plate on a large muscle fibre, while the other formed a rudimentary plate on the end of a thin neuro-muscular spindle fibre.

Perroncito (1902)(47) in a later paper, described an entirely new system of very fine non-myelinated fibrils which pass to the end-plates of lizard muscle. In the sheath of Henle, with the medullated fibres, he found usually one, but sometimes more than one of these fine fibrils, and throughout their course found them non-myelinated, nor did they have any connection with the myelinated fibres as far as he examined them. They passed into the Rouget plate and lay external to the ramifications of the myelinated fibre, forming a very complicated network. Fine branches of this network appeared to pass into the granular sole. He did not know whether they passed out to any other destination, or if they formed connections with/

with the terminal ramifications of the myelinated fibre. In one case, which unfortunately he did not figure, he believed there was a connection between these fibrils and the fibres supplying the blood vessel wall. Perroncito put forward no hypothesis as to the physiological significance, but felt assured that they were quite distinct from the myelinated system.

In relation to Kulchitsky's work to be dealt with later in detail, Perroncito's observations, in this later paper, on the terminaisons-en-grappes in lizard muscle are interesting. He never found these endings on the same muscle fibre with a Rouget plate; he observed all stages between en-grappes endings and Rouget plates; he found cases in which one or more non-myelinated fibres arising from a Rouget plate passed to another fibre to form an en-grappestermination; he stated that the en-grappes endings were constantly found on muscle fibres which were thin, rich in sarcoplasm, and showing a well-marked longitudinal striation; he regarded these muscle fibres as incompletely developed, large clear fibres. While he found two en-grappes endings on one muscle fibre, he never found two Rouget plates on one muscle fibre. He described also his fine fibrils passing out into the terminaisons-en-grappes and he was definitely of the opinion that they were of motor function.

Ceccherelli (1902)(19) whose paper I have not seen, worked on the nerve endings in the tongue of the frog, and a review of this paper was given by Fusari (23). At the base of the tongue he/

he found the endings to be like those in the limb muscles, typical Kuhne endings, while in the middle of the tongue, the endings were more like the en-grappes terminations, and at the tip, definite en-grappes terminations with a plexus of fibres. In the perimysium he described a non-myelinated plexus with fibres in definite connection with the plexus in the muscle and giving branches direct to terminaisons-en-grappes.

Grabower (1902)(28) made investigations on human muscle and confirmed many of the observations that had been made in lower animals. He was unable to classify the nerve fibres into three types as Bremer had done, as he found that they merged into one another and could not be definitely classed. It is of interest that he seldom found a non-myelinated fibre which ran for any distance without appearing as a branch of a medullated fibre, and concluded that it was always derived from the myelinated fibres. He described the two types of endings - end-plates and end-umbels - but found many transitional forms. The end-plates, usually though not always, had a medullated supply, and the end-umbels a non-medullated, but as these non-myelinated fibres were looked upon by him as branches of myelinated ones, it is only the mode of termination that is different, the somatic origin of the fibre being the same in both cases. The end-umbels correspond to the terminaisons-en-grappes of Tschiriew, in that there is always present a small amount of granular substance, and have several branches of a fibre entering them. Grabower also described one muscle fibre having two endings, and in some cases/

cases non-myelinated fibres entered with the myelinated into the formation of the same plate. Unfortunately these observations are of little help in the solution of the present question of sympathetic innervation of muscle.

Gemelli (1906)(25) published a paper which I have been unable to see on the nerve endings in lizard muscle. The account given here is based on a review by Fusari (24). Gemelli used a modification of the rapid method of Golgi and confirmed the existence of Perroncito's fibres, but placed an altogether different significance on them in view of certain differences. In his preparations he found the arborisation of the medullated fibre in the end-plate to be formed of very fine neuro-fibrils with elongated thickenings. The non-myelinated Perroncito fibres accompanying the myelinated fibres inside the sheath of Henle, divided into very fine branches some of which he stated were connected with and ran definitely into the myelinated ramifications. On this account he excluded the possibility that Perroncito's fibres were of sympathetic origin. In the figure given by Boeke (9) of Gemelli's work, this point seems clear, but it is just possible that it is open to another interpretation, that the Perroncito fibres, which while appearing to pass definitely into the end ramifications of the myelinated fibre, may pass just above or below these ramifications and not into them as Gemelli described. It is impossible to do more than suggest this explanation without an extremely careful examination of Gemelli's specimens.

In/

In all the previous work that has been described up to this point, the gold chloride method had been used, unless otherwise stated. Boeke (1909)(7) first used the silver impregnation method of Bielschowsky in his work on the development, form, and connection with the muscle fibre, of the motor end-plate. His work therefore is unlike the older work in that his material was sectioned, not teased, and then examined.

The appearance of the motor end-plate of the reptilian muscle and mammalian muscle which he examined, was strikingly different from the appearance with the gold chloride or methylene blue method. The myelinated nerve, proximal to its termination, showed the axon cylinder as a collection of neurofibrils closely packed together and passing into the end-plate without the characteristic narrowing seen in the gold chloride preparations. The form of the end ramifications is also very different. Instead of the very fine branches ending in deeply stained knob- or button-like structures, he found a very complicated network of neuro-fibrils, showing a great deal of anastomosis between the fibrils, and some of the neurofibrils passed out from the network to form simple end-loops and more complicated end-nets with anastomosing fibrils filling up the enclosed loops. The nuclei of the granular sole plate are very clearly shown. In his slides showing profile or transverse cross sections, he clearly demonstrated the hypolemmal position of the plates. This method has therefore the great advantage that the structure of the plate is not obscured, as it is in the gold chloride method with very darkly stained knob-like structures, and the exact position of the termination of/

of any fine non-myelinated fibre accompanying the myelinated fibre can be clearly visible.

In this paper also Boeke described his periterminal network. In this short survey I shall use the account he gave in a lecture to London University (1921)(11). Here he stated that he thought the neuro-fibrils the actual conductors of nerve impulses, but that up to the present they could not be traced in the end-plates further than the granular sarcoplasm of the sole-plate. In amphioxus he showed what appeared to be direct connection of the neuro-fibrils with the anisotropous discs of the sarcostyles. In other vertebrates with well stained Bielschowsky preparations, he described a fine reticulum in the sarcoplasm, joining up with the neuro-fibrillar network. This he called the periterminal network and thought it to be the anatomical basis of Langley's receptive substance. This periterminal protoplasmic network seemed to pass to the anisotropous discs of the sarcostyles.

The periterminal network he also found in connection with his accessory end-plates now to be described.

In April, 1909, Boeke (6) first described his system of accessory non-myelinated fibres in striped muscle. In October, 1909, he (7) elaborated his statements about them. After reviewing the work of Perroncito, Huber and De Witt, Ruffini, Crevatin, Grabower, and Gemelli he concluded that the existence of accessory non-myelinated fibres passing into motor end-plates could not be substantiated from these workers.

In/

In the muscle of the developing Talpa, human embryo, young mouse and adult lizard, he found very fine non-myelinated fibres, passing either with the myelinated fibre, or from some other course into the end-plate and there forming little end-rings or end-nets. These fibres he never found to have any connections with the branches of the myelinated fibres, both inside the plate and outside the plate. In some cases the accessory end-net or ring was completely separate from the motor end-plate on a granular sole of its own. Transitional stages between this separate accessory ending and the well-known motor ending he never found. That they were hypolemmal he definitely demonstrated, and considered them of motor function. Here he first suggested the sympathetic origin of these fibres, with either a trophic or tonic function. He left it an open question whether they were identical with the Perroncito-Gemelli fibres or not.

In 1910 Boeke (8) in a short note referred to the work of Botezat on avian muscle, but was not convinced of the independence of the fibres described by this worker. Boeke found his accessory fibres and end-plates in the pectoral muscles of the starling (Sturnus.) It is most interesting, in view of Hunter's hypothesis of the two types of muscle fibre, that Boeke's figures here in the pectoral muscles of the bird show definitely the accessory ending on the same muscle fibre as the motor plate, as he had demonstrated elsewhere. The hypolemmal position of these/

these accessory endings, Boeke maintained, made it quite easy to distinguish them from the "atypical motor endings" of Retzius which Huber showed to be epilemmal and thought them sensory.

Boeke, (9) again in 1913, reviewed the question of the identity of the Perroncito-Gemelli fibres with his accessory fibres. He had been unable with the gold chloride and methylene blue methods in lizard muscle to show any connection between the fine fibre accompanying the medullated fibre with the end ramifications of the large fibre. With the Bielschowsky method he had never seen a connection of this sort. He gave then a number of points in which his accessory fibres differed from the Perroncito-Gemelli fibres. This is of special interest in relation to confirmation of Boeke's results with the gold chloride method which I have attempted.

(1) The accessory fibres are single in the majority of cases, while Perroncito figures many where there are two or more.

(2) The accessory fibres do not run inside, but outside the sheath of Henle, and often enter the plate from a different direction from the medullated fibre.

(3) The accessory fibres never show connections with the end-ramifications of medullated fibres in the motor plate, but end in quite independent end-rings and nets; this is true for all his specimens of both young and adult muscle.

(4) While the form of the Rouget plate varies considerably, both in individual muscles and animals, the end-net or ring of the accessory fibres remains remarkably constant in form. This form he states is very like the endings in smooth muscle cells.

(5) The endings of accessory fibres are found, unlike the Perroncito-Gemelli fibres, in separate sole plates of their own, quite removed from the Rouget plates. These are not the ultraterminal fibres, (branches of medullated fibres), although Boeke also occasionally saw this type of fibre.

(6) The independence of the accessory system is conclusively proved by degeneration methods after section of the somatic fibres. In the eye muscles after section of the nerve close to the brain stem only the remains of myelinated fibres were found, but the accessory fibres were intact. So far as Boeke was aware this had never been attempted for the Perroncito-Gemelli fibres. From this result, Boeke claims that his thin fibres must be of sympathetic or autonomic origin.

In a further account of degeneration results Boeke, (1916-17) (10), described experiments on the eye muscles, which have given rise to a great deal of criticism. The third nerve was cut close to the brain stem, and 3-5 days allowed for myelinated fibres to degenerate. By Bielschowsky's method the accessory fibres/

fibres and their end-plates in eye muscles were not found to have degenerated but were normal in appearance. Again after three weeks the muscles were examined. Boeke found a great many of the accessory fibres and endings had now disappeared. This last finding is the one subjected to criticism. Boeke's conclusion from his experiments was that these thin fibres which degenerated after 3 weeks were cranial autonomic and those which did not degenerate were sympathetic fibres entering the muscle by way of the carotid artery. In converse experiments, after removal of the Superior Cervical Ganglian, Boeke thought that the number of accessory fibres was less than normal.

With Dusser de Barenne, Boeke (12) in 1919 described experiments in the cat, where the 6, 7, 8, and 9th anterior and posterior spinal roots were cut inside the dura and the muscle of the 7th intercostal space examined one month later. The staining gave excellent results. No trace of the degenerated neuro-fibrils of the myelinated fibres could be found; but fine non-myelinated fibres were found, quite intact, with end-nets and rings, occurring either at the end of the fibres or from fine side branches. In some cases the ending was more complex, with a number of end-nets close to one another, but this was always much more delicately built than the Rouget end-plate. Granular sole-plates and collections of nuclei suggested their hypolemmal position. These results were exactly similar to Boeke's eye experiments.

Regarding/

Regarding the length of time after section of the nerves, they state that while after 3 to 4 days all medullated motor terminations have disappeared, up to 14 days it is possible to find seemingly intact non-myelinated fibres in the cut nerves. But after a month all myelinated and non-myelinated fibres in the cut nerve have disappeared, so they conclude that the non-myelinated fibres found intact after a month, have a different origin - namely sympathetic.

Botezat (1910)(17) in a note stated that in 1906 (16) working on the muscles of birds, and using the Golgi method, he found thin non-myelinated fibres passing with the myelinated fibres to have endings on the muscle fibre. These he thought were hypolemmal and often occurred in connection with the motor plate, showing clearly that they were found on the same muscle fibre. From his figures it is not definitely clear that he was dealing with a separate set of fibres, but in some cases with collateral fibres, merely branches of the medullated fibres.

Agduhr (1918)(3) cut all the spinal roots forming the cervical plexus in the cat proximal to the Rami communicantes and found after periods of 5-10 days in the interossei muscles remains of medullated fibres or their sheath but a large number of non-myelinated were intact. These he therefore believed to be sympathetic and post-ganglionic. These fibres ended in small simple end-loops in many cases, and were found on the muscle fibres, in the region of the myelinated plates, or/

or, more commonly, distant from these, and also on the muscle fibres of neuro-muscular spindles. The majority of the sympathetic endings he thought hypolemmal, though some he thought were epilemmal. Among the sympathetic plates he found examples, which occurred away from the site of Rouget plates and which had a large extension on the muscle fibre, composed of branches ending in end-loops, more like the motor plates, but distinguishable from them by their delicacy. He shows an example of a single muscle fibre having a degenerated Rouget plate and an intact sympathetic plate.

Agduhr,(1919)(4) described a histo-experimental study which he made in an attempt to confirm several interesting suggestions that arose from a previous experimental study. In this earlier work he found that if maximal stimulations are given to two spinal nerve roots supplying a single muscle and measurement made of the force of contraction, this force is always less than the sum of the contraction forces when the roots are stimulated separately. This immediately suggested to him an overlapping in the muscle of the two spinal innervations, and pointed to the same muscle fibre being plurisegmentally innervated.

He chose such a muscle in the cat or rabbit as the M. Flexor Digitorum Sublimis, which is innervated mainly from the 8th Cervical and 1st Thoracic spinal roots, and slightly also from the 7th cervical. Two of the roots were then cut at different times and allowed to degenerate while one was left intact/

intact. In this way he found the appropriate lengths of time to allow for degeneration, so that the fibres from the different roots, showed either a marked degeneration, a definitely less marked degeneration, or were normal. In this way he found a number of muscle fibres to have two end-plates, one in either stage of degeneration and one normal. Thus in the M. Flexor Digitorum Sublimis, he found a large number of individual muscle fibres having end-plates derived from the 8th cervical and Ist thoracic roots, and a few muscle fibres having end-plates from the 7th and 8th cervical roots. In some cases he found a single muscle fibre with three end_plates respectively from the 7th and 8th cervical and 1st thoracic roots.

In this way, he seems to prove conclusively that for the limb muscles at least the time honoured view of one motor end-plate on each muscle fibre does not hold good, but that a very large number of the muscle fibres are individually pluri-segmentally innervated. Only one argument it seems to me can be brought against his experiments, and that is that the proof would have been more conclusive if the anterior roots alone had been cut, so that possible sensory terminations on the ordinary muscle fibres would have been left intact. He however seems to show a hypolemmal position for all his plates by an accumulation of sarcoplasm and nuclei at the site of the degenerating plate. It is of interest that he shows no muscle fibres with two plates in the same stage of degeneration, or two normal plates. He thought that the flexor muscles showed more/

more pluri-segmentally innervated fibres than the extensors, but could not be definite on this point.

Kulchitsky (1924)(35) using Ranvier's gold chloride method carried out an investigation of the nerve endings in the muscles of the snake (Python). He described two types of terminations - Type I, the usual Rouget end-plate formed from a myelinated nerve, and type II, an elongated simpler form, the terminaisons-en-grappes of the older workers, formed from a non-myelinated nerve fibre. These two systems Kulchitsky maintained were entirely separate.

Endings of Type I were compactly built and were typical Rouget plates with a well-marked granular sole-plate and hypolemmal position. The entering fibres he described as always myelinated. He could find no other system of fibres in or near the end-plates of this type, such as Perroncito described, and thought that in snakes it did not exist. The ultra-terminal fibre of Ruffini and Perroncito, he regarded from his material as simply a collateral branch of the myelinated fibre, which forms a small plate near the Rouget, and more rarely on a neighbouring fibre. It is unfortunate that in his fig. 9 he called one of these collateral plates an "accessory" motor plate, using the term in an entirely different sense from Boeke. On this question of the ultra-terminal fibre, he wrote, doubtless with reliable information, "It is interesting that the authors (Perroncito, Boeke) who formerly confirmed the existence of ultra-terminal fibres as normal, at the present time/

time consider them as only rare exceptions."

The endings of Type II were usually elongated and were constantly formed from non-myelinated fibres. Tschiriew, in his original paper on these terminaisons-en-grappes, and others had described intermediate forms between these and the Rouget plates but Kulchitsky definitely stated that he never saw any of these transitional forms. It is perhaps unfortunate, in view of the older idea that these en-grappes endings were developmental forms, that Kulchitsky did not state whether his material was adult or not. I have had the pleasure of examining some of his specimens, and they do very forcibly suggest an entirely different origin for the two types I and II. The position of Type II, hypolemmal or epilemmal was, he stated, as yet doubtful, but thought it probably epilemmal. He, in spite of this fact, maintained that they were motor endings.

The thin non-myelinated fibres never showed any signs of being branches of the medullated fibres, but formed a plexus in the endomysium where they ended in free terminations, and in the muscle where they formed the endings of Type II. In connection with this system, he found a number of small unipolar ganglion cells, from which after short single processes both branches appeared to be non-myelinated. In one case, fig. 12, he showed a ganglion cell actually in relation to an ending of Type II. The great differences from the myelinated fibres made Kulchitsky suggest that this second system, with its en-grappes endings, is of sympathetic nature.

In relation to Boeke's work, it is of great interest that Kulchitsky/

Kulchitsky stated very definitely that the two types of nerve ending never were found on the same muscle fibre. He however made no statement concerning the existence of two types of muscular fibre, as did Hunter. From his figs. 7 and 8 it would appear as though the endings of Type II were found on muscle fibres whose diameter is smaller than those having endings of Type I. On this fact Kulchitsky makes no comment, and his fig. 9 does not show this difference so markedly.

In a later paper Kulchitsky (1924)(36) made observations on the nerve endings of the frog, using the methylene blue method in conjunction with the gold chloride method. He described three types of nerve fibre, (a) myelinated fibres which often lost their myelin sheath before termination, (b) non-myelinated fibres; some of these were branches from myelinated fibres usually at nodes of Ranvier while others were distinctly non-myelinated for a long course and formed plexuses and were according to Kulchitsky of cerebro-spinal origin also, (c) non-myelinated nerve fibres of the sympathetic system. This last group of fibres and their endings are not dealt with in this paper, but were to be communicated in a subsequent paper.

The motor terminations he held to be hypolemmal and the sensory epilemmal. He described a thin plasmatic layer enclosing the axons entering the muscle and suggested that it was analogous to the granular sole of the lizards, birds and mammals. Again he insisted emphatically that each muscle fibre received only/

only one motor ending. The "atypical endings" of Retzius he stated were sensory in nature as Huber (31) first maintained. The non-myelinated fibres as well as the myelinated formed the sensory endings of the terminal threads with leaf-like expansions. These endings were sometimes quite simple, two or three expansions, or sometimes much more complicated, suggesting an end-plate. Several of these sensory endings could be found on one muscle fibre as others had described. He thought it possible that the sensory terminal threads also had a plasmatic layer. Some of the non-myelinated fibres he found to end in the connective tissue of the muscle.

In view of Hunter's theory, it is unfortunate that Kulchitsky made no reference to the thin granular muscle fibres which do undoubtedly exist in the frog, nor to the nerve terminations which could be found on these thin muscle fibres.

A note of comment must be made on the different views which Kulchitsky expresses in his two papers. In one he maintains the epilemmal situation to be characteristic of sensory endings, but in the other he maintains his Type II to be motor. Again a number of his non-myelinated fibres in the frog are not seen over a long distance to have connections with the myelinated fibres and are classed as cerebro-spinal sensory fibres, while, in the snake the non-myelinated system, also ending in epilemmal endings, is classed as motor sympathetic fibres.

Dart (1924)(21) in a paper on the double innervation of striped muscle, based in part on the examination of Kulchitsky's specimens, made a number of statements which cannot be borne out by Kulchitsky's own description. He stated that one muscle fibre received several endings of different types and that the so-called non-myelinated fibres could be divided into two categories, (1) absolutely non-myelinated, ending in terminaisons-en-grappes, which he considered sympathetic sensory, and (2) finely myelinated fibres ending in small plates like myelinated plates but without any granular substance, which he considered sympathetic-motor. These observations, completely at variance with Kulchitsky's, make it difficult to lay any weight on them, and while his scheme of the various connections of the sympathetic fibres is interesting, too many points in it yet need confirmation to warrant its acceptance.

Kuntz and Korper (1924)(37) made an attempt to confirm some of Boeke degeneration experiments. In their control material they were successful in finding Boeke's accessory fibres and end-nets with the Pyridine silver method, and for the first time, with the gold chloride method. They do not give details of the gold chloride specimens as to how far they were like those of the silver method. Four weeks after section of the 7th, 8th and 9th Thoracic nerves proximal to the Rami Communicantes they examined the 8th intercostal muscle in dogs and found degenerated myelinated fibres but Boeke's accessory fibres and end-nets intact. In another dog the mandibular nerve was/

was cut intracranially and the Masseter and Pterygoid muscles examined, where the same results were found. Boeke's results were therefore confirmed for the first time by the Gold Chloride method. While no specific mention is made of the fact that the accessory endings were found on the same fibre, such may, I think, be presumed, as such a wide difference, as the two types of endings being on different muscle fibres, would have been mentioned, if such had been the case.

Hunter (1924)(33) stated at the beginning of his review of the histological work on sympathetic endings in muscle, that Grützner's theory must be substantiated by some muscle fibres having a medullated nerve supply and other fibres only a non-medullated. He accepted Kulchitsky's view that medullated and non-medullated fibres never ended on the same muscle fibre, and also that the non-myelinated fibres ending in the terminaisons-en-grappes were of sympathetic origin. This was an entirely new view of their origin, and it seems to me in relation to Hunter's widespread application of this work, that two points must be kept in mind, firstly, that this work was done on snake muscle and not on mammalian, and secondly, that <u>no</u> degeneration experiments were made to prove the sympathetic nature of the fibres. Hunter quoted Tschiriew and Perroncito in support of the terminaisons-en-grappes being on different muscle fibres from the Rouget plates; they, however, did not look upon them as being sympathetic endings.

Of Boeke's work Hunter gave a very severe criticism. The

fact/

fact however remains that Boeke attempted and proved to his satisfaction by degeneration methods that his system of fibres were of sympathetic origin, while no degeneration experiments have yet been published in proof of Hunter's contention. Hunter cited Boeke's experiment of cutting one of the nerves to the extrinsic muscles of the eye close to the brain stem and examining the muscle after periods of 3-5 days and 3 weeks. The difference in the number of accessory fibres found, large numbers after 3-4 days and relatively few after 3 weeks, Boeke explained by saying that after the short period both sympathetic and cranial autonomic fibres were present, and that after 3 weeks only the sympathetic fibres were present. This is open to criticism as where could the autonomic fibres have been cut off from their ganglion cells by his operation? Wilson (1921)(58) suggested that after a short period (3-5 days) of degeneration some of the thin fibres were really the myelinated fibres which had slowly degenerated and lost the myelin. This objection must carry a certain amount of weight. The whole question of the eye muscles is complicated by the fact that there are very numerous small endings of the type described by Retzius and shown by Huber to be sensory in nature. This may lead to some confusion in the interpretation of results, but it is important that Agduhr found that the sensory fibres in the limb muscles degenerate more quickly than the motor fibres, and by analogy it seems probable that these sensory fibres in the eye muscles would also degenerate more quickly

and/

and therefore would not complicate the question so much as might be expected. Hunter stated "that the anomalies in Boeke's interpretation of the accessory system undermine his contention that every individual muscle fibre receives both a sympathetic and somatic ending."

It seems to me possible to explain the differences obtained after a short and longer period of degeneration in another way. Presuming that the staining of the two lots of material was equally good, the existence of fewer accessory fibres after 3 weeks, may be due to the fact that with the inactivity of the muscle some atrophy of the sympathetic fibres may take place, not on account of their being cut off from their nerve cells, but simply due to the inactivity of the muscle.

Boeke's converse experiment of excising the superior cervical ganglion, Hunter stated, did not establish the fact that all the accessory fibres were sympathetic. It is true that Boeke did not find the whole system to disappear, but only thought them fewer in number. This, it seems to me, is all that could be expected in such a complicated plexus of fibres, where connections are found with the perivascular plexus and where the existence of some small ganglion cells peripheral to the superior cervical ganglion may yet be demonstrated.

The intercostal experiments appear to me to afford a much easier field of investigation and here the sympathetic nature/

nature of the accessory fibres seems to have been definitely proved. Hunter, however, advances the objection that this is only proven for the muscle as a whole and not for the individual fibres of the muscle. The period of degeneration (1 month) makes it impossible to show the double innervation of each fibre, but as Boeke had already shown in a very large number of his figures, that the accessory fibre ended in the same sole plate as the myelinated fibres, (and some of these figures were from intercostal muscles), one can surely definitely state that some muscle fibres at least are innervated by both systems. Histologically it would be extremely difficult to show the double innervation, somatic and sympathetic, on every muscle fibre, but some individual fibres are certainly proved, in my reading of Boeke's work, to be doubly innervated.

Of Agduhr's work, Hunter gave a short account and criticised it on the ground that as Agduhr stated his "accessory" plates approached in form to those of the motor plate, and were by Hunter thought to be non-myelinated collateral branches of myelinated fibres which had not yet degenerated. The particular case he cited was found after 5 days' degeneration. This, however, is only one particular case, and after a period of 10 days similar appearances were found which ruled out the possibility of a somatic fibre ~~and~~ on account of its evenness of staining and completeness. Agduhr stated in reference to his fig. 2 - "An intact sympathetic nerve with a simple loop formation terminates within the region of the motor plate. There/

There are thus instances of sympathetic nerve fibres in the musculature of the extremity, that terminate within the region of a motor plate." Clearly this means a double innervation of these fibres at least, leaving out of account if necessary the larger accessory plates whose sympathetic nature Hunter questions, although Agduhr remarked "it is as a rule easy to distinguish even in preparations where all the nerves are intact between motor and sympathetic terminal plates of nerves."

The histological work, that Hunter brings forward to prove his contention that the sympathetic and somatic fibres do not end on the same muscle fibre, has not yet been published and only a short note of it is given in his lectures. Hunter and Latham examined the mode of ending of the nerves in the limb muscles of the goat and fowl, which were used by Hunter and Royle in their experimental work. "In no case were they able to find a muscle fibre receiving both kinds of nerves." Latham, however, distinguished hypolemmal sympathetic nerve on the slender muscle fibres. As Hunter and Royle do not state that they found any variation in size in the normal muscle in the goat, and state definitely that very little variation could be seen in the normal muscle of the fowl, it is doubtful to what Latham refers when he speaks of slender muscle fibres.

---

4. On Neuro-muscular Spindles.

Of the older descriptions of the neuro-muscular spindles no mention will be made here. At that time these organs were considered to be germinating centres for muscle fibres and very little was known of the nerve fibres connected with them. It was only when fuller descriptions were given of their nerve terminations that their sensory function became evident.

Sherrington (1894)(55) before giving his own observations gave a review which may be consulted for the older work. In the experiments after section of the motor nerve roots (anterior) he always found that every spindle seen still possessed perfectly sound myelinated nerve fibres. He therefore proved them to be sensory organs. From his own observations in cats and monkeys he gave the following account. The lamellated capsule completely enclosed the periaxial lymph space through which the muscle fibres of the spindle ran. At the proximal polar region these primitive muscle fibres, 2-12 in number, were of the "red" (Ranvier) or "trube" (Grützner) variety; as they penetrate into the organ, they split longitudinally into 2 or 3 daughter fibres. There they showed numerous nuclei in the thickness of the fibre and often the marginal sheet of the substance of the fibre was alone striated. In the equatorial region the surface of the fibre was encrusted with a sheath of nuclei. At the distal polar region the muscle fibres resembled in appearance those of the proximal polar region.

The/

The nerve fibres of the posterior root passing to the spindle were large myelinated fibres, 7-17μ in diameter. They showed a very well-marked sheath of Henle. Before they penetrated the capsule the internodal segments were from 600-900μ long. While inside the spindle this distance was shortened immediately to 80-130μ. Inside the sheath the fibres usually divided and became less thickly myelinated although their diameter remained the same or slightly increased. The nerve fibres showed a serpentine course and ended in wide flattened expansions, which Sherrington took to be the annulo-spiral ending of Ruffini.

It is most interesting that Sherrington with the gold chloride method could not satisfy himself of the existence of motor end-plates. But after removing a piece of the sciatic nerve, preventing regeneration and waiting 150 days, he found all the ordinary muscle fibres of the gastrocnemius degenerated while the intra-fusal muscle fibres were quite normal. To quote his conclusion "The intrafusal muscle fibres seem in regard to their nutrition to be largely independent both of the afferent and of the efferent nerves of the muscle, if one may judge by absence of obvious degeneration in them for five months after total denervation."

Sherrington did not observe any of the smaller afferent nerve fibres entering a muscle spindle. He did not find any spindles in the extrinsic eye muscles nor in the intrinsic muscles of the tongue.

Ruffini/

Ruffini* (1898)(52) gave a very minute account of the structure of the muscle spindles of mammals with special reference to the cat. The description of the intrafusal muscle fibres and their endings alone need be considered here. He described the muscle fibres as showing very well-marked striation and nuclei more abundant than in the ordinary fibres. The nerve endings in the spindles he divided into three types: the primary ending was formed from a very large medullated fibre, which divided into 2 or 3 non-myelinated branches, and these flattened and wound themselves round the muscle fibre in the form of rings or spirals - the annular or spiral endings, or a mixed form the annulo-spiral: the second ending was formed from medullated fibres, never so large as those of the primary ending, and were found on either side of the annular or spiral ending. The nerve fibres to this ending may pass in with the primary fibre, but often enter the spindle from another nerve branch. The ending itself he described as having varicosities and suggesting sprays of flowers. His third type of ending, or plate-like termination is of special interest in view of more recent work. These he stated were very variable in size and form, some being larger than and some smaller than the Rouget plates in neighbouring ordinary muscle fibres. Ruffini could find no granular sole plate nor eminence of Doyère. These endings are formed from very delicate nerve fibres, which he thought branches of larger fibres; they could be found entering with the first to the primary

or/

or secondary endings, but sometimes came from a different nerve branch and entered the spindle by themselves. He found no branching of these fine fibres inside the spindle, so that each ending had a fibre of its own. From a comparison of a large number of spindles, Ruffini stated that the primary and plate-like endings are constantly present. In summing up he concluded that there were no motor endings on the spindle muscle fibres as on analysis of the plate-like endings he thought them sensory. He never found a fibre from a motor nerve bundle passing to the spindle.

Golgi (1903)(26) described the muscle fibres of the spindle to be distinguishable from the ordinary muscle fibres, by three points, (1) they were thinner, (2) constantly furnished with much more abundant nuclei of the sarcolemma, (3) throughout their whole length many of the nuclei lay inside the muscle fibre and had round them granular protoplasm. They were described by him, as being like the embryonic ordinary fibres.

Perroncito, (46) as already mentioned, described fibres passing to the muscle spindle in lizards which could hardly be other than motor. He was of the opinion that the muscle spindle had motor as well as sensory nerve connections. In his later paper (47), he found his very fine non-myelinated fibres to pass into the muscle spindles, as in this situation they were easily demonstrated. His figures show a very complex network of these fibres both in the capsule and inside it. The ultimate connections in this organ he could not determine but thought that/

that in some cases they were in contact with the muscle fibres. These thin non-myelinated fibres entered the spindle in the Henle sheath of the medullated fibre, and not with the blood vessels.

Golgi (1919)(27) was also of opinion that the muscle fibres in the spindles had both sensory and motor terminations upon them.

Dogiel (1902)(22) described the nerve endings of the muscle spindles. He found one or more thick medullated fibre passing into the capsule and ending in non-myelinated branches, round the spindle muscle fibres as Ruffini described. In addition to these, two or three thin medullated fibres formed endings, either inside or outside the capsule, which were of various forms, but were all small and plate-like. These he considered to be motor in function. He also found passing in along the blood vessels sympathetic nerve fibres, as Huber and De Witt(32) described. They evidently were connected only with the blood vessels.

Cilimbaris (1910)(20) made investigations on the muscle spindles of the extrinsic eye muscles of various animals. He found them present in these muscles in the sheep, roe, stag, goat, cow, and wild boar, but absent in the horse, pig, dog, cat, fox, rabbit, hare and rat. The spindle muscle fibres were found to be often small but were sometimes large, and both sarcoplasm-rich and sarcoplasm-poor fibres were seen in the spindles. Inside the capsule of the spindle the muscle fibres divide dichotomously/

dichotomously and these smaller daughter fibres united together at points to form a network. At the site of the sensory nerve ending, he described inside the muscle fibre, globules (bläschen). These were only found in this situation and were related to the setting up of the afferent impulses in the sensory endings. The nuclei of the spindle muscle fibres were often placed more centrally than in ordinary skeletal fibres, but the contractile substance was the same as in ordinary fibres.

Of the nerve fibres passing to the spindles the majority were medullated. He believed that in isolated cases he had seen non-myelinated fibres which went to the spindles but could not be definite on this point, and in these few cases he thought them vasomotor fibres. While he held that both motor and sensory fibres passed to the spindles he could not agree with those who said that the thick medullated fibres were sensory and the thin medullated fibres motor. He found the calibre of the fibres subject to great variation, both very thick and extremely fine; typical motor end-plates were sometimes found formed by a thick medullated fibre and sometimes by a thin fibre. In the same way, the sensory ending could be formed from either large or small medullated fibres. He was convinced that there was no difference in the motor supply of ordinary muscle fibres and the spindle muscle fibres. In further proof of this he cited cases, like those of Perroncito, where a motor fibre divided into two branches, one of which ended inside the spindle on a spindle muscle fibre, while the other ended on an ordinary muscle fibre.

Also/

Also he described a motor fibre inside the spindle dividing into two branches which ended one on intrafusal muscle fibre and the other, after passing out, on an ordinary muscle fibre. He never found more end-plates in the spindle than there were muscle fibres and no case of one muscle fibre with two motor plates was seen. These motor end-plates he stated in no way varied from the motor end-plates on the ordinary muscle fibres. He noted the presence of the ultraterminal fibre of Ruffini in a number of his end-plates on the spindle muscle fibres, and it often passed outside the spindle to end on the ordinary muscle fibre.

The sensory endings described by Cilimbaris were like those of others, only differing in the "bläschen" formations found in the muscle fibres where the sensory endings were situated as already stated. He pointed out that while the spindle muscle fibres had both a sensory and a motor termination upon them, there was always a termination-free part of the muscle fibre between the two.

Sutton (1915)(56) found muscle spindles in the extrinsic eye muscles of embryo pigs while Cilimbaris could not find them in those muscles in the adult of the domestic pig, although he did see them in the wild boar. In the earlier stages a very complicated network of fibres was found in the muscle, but as the embryo developed, the plexus formation became less and less, until large branching fibres were left and these became myelinated.

He/

He found the early spindle muscle fibres at first to be striated throughout their whole length, but later this striation was lost in the intracapsular part. It is also interesting that he described the capsule as a late development.

Kulchitsky (1924)(35) described the muscle spindles in Python muscle and divided them into a simple form and a more complex one, the small and large spindles. He maintained that the spindle muscle fibres retained their cross striation throughout their whole length and did not show any change in thickness inside the capsule. Usually one medullated fibre was found entering the capsule, and after losing its myelin formed ribbon-like or spiral rings embracing the muscle fibre. The endings he thought were on the surface of the fibre, and were sensory. Besides the myelinated nerve fibres, Kulchitsky found non-myelinated fibres which he thought were sympathetic and which ended in terminaisons-en-grappes on the muscle fibre of the spindle. These he found outside the limit of the capsule and several endings could be found on one spindle muscle fibre, but he also described non-myelinated fibres passing inside the capsule to end on the muscle fibres; these however he did not show in his figures 10 and 11 to end in terminaisons-en-grappes, although he believed they did end in this way. These non-myelinated fibres he considered to be quite separate from the myelinated fibres, as he described in the rest of the muscle, and to be motor in function.

In his later paper (36) on the innervation of Frog's muscle/

muscle he made no reference to muscle spindles.

Dart (1924)(21) from his observations on Kulchitsky's specimens, made several statements which cannot be correlated with Kulchitsky's own description. In the short oval capsulated spindle the medullated fibre is said to divide into non-myelinated branches and end in plate-like forms within the capsule. The sympathetic fibres ended on the muscle fibres of the spindle in what he described as motor sympathetic endings, contrasting them with the sympathetic sensory or en-grappes ending.

The resistance to atrophy described by Sherrington, he thought might be accounted for by (a) the preservation of primitive characters of the muscle fibres, and (b) the very rich sympathetic motor innervation. Dart also remarked that he awaited confirmation of coarsely myelinated motor fibres going to a spindle as Perroncito described.

PART III.

ORIGINAL WORK.

I. On the Striated Muscle of the Hedgehog.

Material.

The fully grown hedgehogs were killed with chloroform and the muscles dissected out as quickly as possible. When the panniculus carnosus was dissected out it could be seen to receive branches from each spinal segment. These branches run across the sheet of muscle on its ventral aspect and give off as they pass out small branches which pass into the muscle. In the substance of the muscle the small nerve bundles always keep a course at right angles to the length of the muscle fibres. All the observations were made on this muscle. Small pieces of the muscle were quickly removed and carried through by the method now to be described.

Method.

This was the Fischer modification of Ranvier's method as used in Golgi's laboratory, and will be given in detail.

1. Immerse the small pieces of tissue in a 25% solution of Pure Formic Acid and depending on their size leave for 10-15 minutes. See that the immersion is complete. During this period, tease the preparation a little to get better penetration. Too much fluid should not be used.

2. Take the pieces quickly from the acid solution and place on a clean duster folded, cover with another fold and press gently to absorb as much acid as possible.
3. Pass to a 1% solution of gold chloride, just sufficient to cover the pieces completely and shake the pieces in it. No iron instrument must be used in this or subsequent baths. Bone pointed forceps or metal forceps covered with paraffin wax must be used. It is advisable to cover the dish with a blue or yellow glass. Leave 20 minutes at the most.
4. Carry out 2 again with clean duster.
5. Pass tissue into a 25% solution Formic Acid. Use just enough to cover the pieces of tissue. Leave in absolute darkness for 24 hours.
6. Proceed as in 2 again.
7. Pass to pure glycerine and leave in ordinary light in a closed vessel. The longer the tissue is left in glycerine the clearer does the preparation become. (Some of my best specimens of frog muscle are five years old)

Cleanliness is paramount in order to get good results, and solutions were made up in double or treble distilled water.

The preparations are made by teasing small pieces of the stained muscle or nerve and mounting these in pure glycerine. The permanent preparations are ringed with a cement, or gold size.

The method of final preparation, that of teasing, has

very/

very definite limitations. The complete continuity of the fine nerve fibres and fibrils must of necessity be sacrificed in many instances in the interests of clarity. The definite establishment of continuity in even one case is therefore of extreme importance and totally outweighs the fact that it has only been seen once. The teasing then while it sacrifices the multitude of fine fibrils in the preparation, does in some few and fortunate specimens, show their course and continuity with great clearness. This method is therefore only capable of showing continuity throughout a short distance of their course, unless in exceptional cases.

The nerve fibres when well stained show intensely purple or black. If therefore a thick nerve fibre is accompanied by a very fine fibre of very much smaller diameter, it is obvious that in one of two positions out of the large number of possible positions which the thin fibre may occupy on the periphery of the large fibre, will it be clearly seen, when viewed from above, (i.e. ↓ •●•). At all the other possible positions below, it will be obscured by the dark thick fibre and above, it is extremely difficult on focussing up to be sure of its existence against the dark thick fibre as it is itself dark. It is therefore possible for a fine accompanying fibre to pass out of sight, and yet retain its independence of the large fibre, unless it can be traced to pass out of sight at a node of/

of Ranvier, at which point only do myelinated fibres in their course give off branches myelinated or non-myelinated.

(a) <u>The Muscle Fibres</u>.

In order to examine the whole length of a single muscle fibre, which is extremely difficult in teased preparations of the Panniculus Carnosus muscle, the small pieces of muscle after being in glycerine for a long period were washed in distilled water and placed in a weak solution of nitric acid to dissociate the different elements. This caused a great shrinkage, but otherwise did not seem to harm the tissue. The single muscle fibres could then be mounted, a number on one slide, in glycerine.

This method of preparation reveals the fact that the muscle fibres are peculiar in that they are all long and fusiform, tapering towards both ends very slowly, from the middle of the fibres. They are unlike the usual striated fibres then which show as parallel sided cylinders for nearly their whole length and only show rounded ends just at their attachment to the tendon. A considerable degree of variation in size of the individual fibres was found, some of the smallest fibres, showing a diameter at their middle of one half or one third of the diameter of the larger fibres at the same point. Therefore where the whole length of a fibre in a teased preparation cannot be followed out, it becomes a matter of great difficulty/

difficulty to decide whether the particular fibre is really a small fibre or the tapering end of a large fibre.

With reference to the plurisegmental innervation of each fibre, it is interesting to note here that the muscle fibres are much longer than is the distance between the segmental nerves as they pass out over the whole muscle. Some of the larger fibres which are also the longest are long enough to have three segmental nerves passing across their length at different points.

The cross striation in the dissociated fibres is not very well marked: this I think is due to the staining, as in some of the other preparations the cross striation is well marked. As to the existence of two types of muscle fibres, the following points may be made. On account of size, two types of fibre could not be distinguished clearly, as all sizes between the largest and the smallest can be found. The degree of longitudinal striation is more interesting. Some of the fibres show a much rougher longitudinal striation than do the others, and while some intermediate grades may be found the two sets might be more clearly differentiated by this than by size. Besides the more deeply stained longitudinal striation these fibres show very deeply stained little elongated masses of sarcoplasm in the centre of which may be seen clear oval structures, the muscle nuclei. These elongated masses may be longer and show nuclei at equal intervals/

intervals in their course. These fibres would therefore appear to have a larger amount of sarcoplasm. Comparing the number of the nuclei in the longitudinally striated fibre, with the number of nuclei in the fibres not showing this marked longitudinal striation, they do not appear to be more numerous in the former type. In the latter fibres, the nuclei show less clearly as the sarcoplasm round them is not deeply stained. This it seems to me raises the question at once as to whether these markedly longitudinally striated fibres are not simply fibres which are more deeply stained. It is necessary to state that in this muscle for a reason to be given presently uniform penetration and therefore staining is very difficult. The cross striation is in these fibres as a rule also very well marked. The size of the deeply stained longitudinally striated fibres varies considerably and while a number of these are of the smaller sizes, one or two of the larger fibres have also been seen in this condition. The total number of these fibres is very small compared with the number of the fibres not showing these peculiarities. By no means all the small fibres show them. In view of the appearance seen in frog muscle to be described later where two different types of fibres are known to exist, it seems to be impossible to say definitely that in the panniculus carnosus two distinct types of fibres exist, but appearances/

appearances suggest that for some reason the sarcoplasm in a few of the smaller fibres stains more deeply and may be present in larger amount than in the other fibres.

The arrangement of the muscle fibres in the sheet of muscle shows that the individual fibres interdigitate with one another so that at any one line drawn transversely across the muscle, one finds all stages of the tapering ends, from the very thin ends up to the large diameter of the middle of the fibre. This makes the muscle very difficult to tease without injuring a large number of the elements, and makes uniform penetration of the staining reagents and consequent uniformity of staining very difficult.

(b) The Constitution of the nerves entering the muscle.

Observations were made of teased nerve bundles as they entered the muscle, or of bundles in the muscle at some distance from their endings. These bundles were stained by the same method. This investigation was made to see if there were any fine non-myelinated fibres present in the intramuscular nerves or nerves about to enter the muscle, and if so, in what numbers were they present. That is, it is an attempt to eliminate a criticism, which might be levelled at the demonstration of non-myelinated fibres near the region of the motor plates of Rouget, that in this situation these non-myelinated fibres are simply small non-myelinated branches of the myelinated fibres in or near this region. The demonstration/

demonstration, then, of a number of fine non-myelinated fibres in the nerve at its entrance into the muscle would in part eliminate this objection, as it is much more reasonable to suppose that the fine non-myelinated fibrils in the region of the motor plate are the continuations of some at least of the fine non-myelinated fibres found in the nerve some distance proximal, than to postulate that they are new branches of the myelinated fibres near the end-plate, and that the non-myelinated fibres found proximally have some other termination before they reach the region of the motor end-plates.

On examining these teased nerves, when they are well stained, the myelinated fibres are deeply stained and their nodes of Ranvier well marked. There is found to be extremely little or no branching of these fibres at the nodes. The great majority of these fibres are large well-myelinated fibres showing some variation in size. Smaller myelinated fibres are also seen in smaller numbers, but easily distinguishable by their nodes from the next type of fibre to be described.

A relatively large number of non-myelinated fibres can be found. These are easily differentiated from the myelinated fibres. They do not have the sharp outlines of the myelinated fibres and show no sign of segmentation at nodes of Ranvier.

They/

They are less deeply stained and vary in size from that of the small myelinated fibres to extremely fine fibrils. They show in their course the characteristic nuclei of the pale fibres. Some of these larger pale fibres divide up into a number of thin fibres which may pass on singly or reunite with their fellows, or unite with other thin pale fibres, showing that their tendency to plexus formation is already present in the nerve. They may be found single accompanying a thick medullated fibre or in groups twisting and twining together. They are clearly distinct from the myelinated fibres and are not found as branches of these fibres.

The same facts could be observed in the smaller intra-muscular nerves, still some distance from the muscle fibres they supplied. The existence then of a separate system of non-myelinated fibres is definitely proved in these nerve bundles.

(c) The Motor Endings.

On even a preliminary examination of the motor endings of the medullated fibres, the variation in size, shape and form is striking. The form of the motor plate in this muscle is very complicated and not unlike type I, as described by Kulchitsky in snake muscle. After long examination of many preparations it has been impossible for me to make any division/

division of these varying forms into definite classes or types, and while some of these variations resemble in some respects the terminaisons-en-grappes it has been impossible to make any separation between these forms and the more typical Rouget plates as all forms intermediate between these two are found not in a few cases but in many.

A more detailed description of some of these variations will now be given.

The medullated fibres as they pass across the muscle fibres, to which they are connected, show some branching at the nodes of Ranvier. It is of interest that while a medullated fibre may in some cases appear not to divide at all but to end in one Rouget Plate which is usually large in size, the fibre may as it passes give off 3 or 4 short branches, all of which end in large plates, and then end itself in a large plate. What relation this has to the numerical method, now in use, of counting the fibres on cross sections of a nerve in determining the functional activity of that nerve, is not quite clear. But if one nerve fibre may either innervate one or five muscle fibres, as it appears, this fact must be taken into account.

Near the point of entrance of the medullated fibre, into the muscle fibre, the myelinated sheath may be lost. In a number of cases this may occur at some distance from the plate. The branches given off by the medullated fibre, which form end-plates may also be non-myelinated, or at least very thinly myelinated/

myelinated, so that some motor end-plates may be found which have a medullated fibre, while others have a non-medullated fibre. But this distinction seems to be of very little value, as these non-myelinated fibres are simply branches of the axon of the medullated fibre, and must have exactly the same origin and function. In some cases where the further course of one of these non-myelinated fibres is obscured, it seems to me unnecessary to suppose that it is a non-myelinated fibre throughout its course, when this form of branching - non-myelinated fibre from myelinated - is so common in all the preparations.

Some of the branches are definitely medullated, though by no means so heavily as the parent fibre. While it is true that in some cases this apparent loss of myelin proximal to the end-plate may be due to stretching, or the teasing of the preparation, in other cases it can be definitely stated not to be due to this mechanical strain.

Examining now the mode of entrance of the fibre into the muscle fibre, in many cases, especially in those plates showing an oval or rounded form, the nerve fibres pass directly under the sarcolemma without dividing. In others, the fibre may divide into two or more branches each of which enters separately into the muscle fibre. This is often seen in the case of two branches and a special form of end-plate to be described later is often associated with it. Some others of these branches are collaterals and will also be described later in their terminations.

A peculiar form of this branching has been observed in some of the specimens. As the two branches pass together to the plate separated only by a small angle, the sheath of Henle appears to be greatly thickened and corrugated with a large number of nuclei. Through this the axon cylinders pass in a spiral fashion. One of this form may be seen in Fig. 13, which however is complicated by the further branching of the fibres to supply other plates. It can however be seen in many cases where only the branches supplying one end-plate are present.

Variations in size of these motor end-plates are marked, some of the endings only occupying approximately one quarter of the area of the largest plates seen. The largest plates lie as a rule in the middle portion of the largest fibres, that is near their point of maximum diameter. They are formed more commonly from nerve fibres which are medullated up to, or very close to their point of entrance into the fibre, and much less commonly from fibres which lose their myelin proximal to their entrance into the muscle fibre. The smaller end-plates are more commonly formed from the non-myelinated branches or from thinner myelinated fibres, but in some cases a quite thick myelinated fibre may form a smaller plate. These smaller plates are often found on muscle fibres of smaller diameter, which are frequently really the tapering ends of a large muscle fibre. Quite often, however, these small plates are seen on the larger muscle fibres where their diameter is still relatively great.

Some/

Some conclusion as to this great variation in size of the motor end-plates may be drawn from an examination of the dissociated muscle fibres described above. This was primarily undertaken in order to be able to follow out easily the whole length of a single muscle fibre and to see if more than one ending could be found on it, with particular reference to Agduhr's statement that the individual fibre is innervated plurisegmentally. Agduhr's specimens from limb muscles show the different segmental plates very close to one another on the muscle fibre. In a muscle such as the panniculus carnosus, no joining together of the spinal roots occurs so that one could not expect to find end-plates close together on the single fibres. In favourable cases two or more end-plates separated by a long distance, have been found on single fibres (fig. 1). Now as very few fibres of the nerve bundles show a longitudinal course unless for a very short distance just proximal to their ending, and the distance between the end-plates is approximately the distance between the segmental nerves, I think it can be safely concluded, that in these cases single muscle fibres are innervated plurisegmentally. Numerous instances were not found but this was doubtless due to the difficulty of keeping the long single fibre intact in the process of dissociation and mounting, and to the fact that while one end of the muscle fibre might be appropriately stained to show an end-plate, the other end was often too deeply or too lightly stained.

In/

In this examination, it is found that the larger motor end-plates are seen near the middle of the fibres while smaller plates are often seen nearer the ends of the fibres. That both the end-plates on the one fibre are motor is proved by their hypolemmal position and granular sole-plates. The plurisegmental motor innervation of the single muscle fibre can therefore be confirmed for at least some of the muscle fibres of this muscle. It differs only from Agduhr's demonstration in that the plates are very far apart on the single fibre.

During these observations it was easy to examine the endings on the more longitudinally striated muscle fibres. I was not successful in finding such a fibre with a plurisegmental innervation, not, I think, because they do not exist, but on account of the very small number of these fibres which could be found, and then followed out for any considerable distance. That these fibres with the well-marked striation were innervated by exactly the same type of nerve fibre as the other muscle fibres, and with the same type of end-plate, could be perfectly definitely established. These fibres received a myelinated fibre, often a very thick one, which is contrary to the idea that these fibres might have only a non-myelinated sympathetic nerve supply.

It is to be regretted that these dissociated preparations do not show the very fine non-myelinated fibres to be described later. This is no doubt due to the method of preparation as it/

it is quite unlikely that such fine fibrils would withstand such treatment.

Great variations in the form of the Rouget end-plates are found. In the hedgehog the terminal ramifications of the axon-cylinder show a very much more complex formation than is seen in many of the other vertebrates - much more complex in my preparations than those seen in lizard, rat, rabbit or human muscle. The branch or branches of the myelinated fibre after passing through the sarcolemma, divides up into fine branches. These branches show in many specimens a great deal of anastomosis, so that an end-net is formed. Parts of these branches, either the terminal points and the points of junction or anastomosis show thickenings, which are very deeply stained. The terminal thickenings are often rounded or knob-like. The degree of branching and anastomosis varies very considerably. In some cases one part of this terminal arborisation is very much more marked and gives the appearance of a central whorl or wheel with radiating spokes, set in the less well-marked branching of the remainder of the terminal ramifications.

In some cases where two branches of nearly equal thickness pass into the end-plate, they each form terminal ramifications which remain discrete in the same sole plate, suggesting the appearance of a double plate. In other cases where the two branches are of unequal thickness, the large branch passes in and forms the large part of the plate, while the fine fibre passes often to the most distal point in the terminal ramifications. It is curious to notice this long course of

the/

the thin branch to reach this point furthest from its starting point.

The shape of the area occupied by the terminal ramifications in the plate is frequently rounded or oval, but many shapes are seen. Elongated forms are fairly common, and less rarely quite irregular forms can be observed. The smaller Rouget plates show similar variations in shape, but much less markedly than the larger plates, as the former are much more commonly rounded or oval.

In all the preparations which were properly stained, a granular sole-plate could be seen extending over a slightly larger area than the terminal ramifications. In some cases it was much more darkly stained than in others, but it was invariably present. At the edge of the granular sole, in a number of cases the nuclei of the sole could be seen, as large clear areas.

As has been mentioned above, no difference at all could be found in these motor plates which lay on the muscle fibres which were markedly longitudinally striated. All that may be said is that the rounded or oval form was seen on them more commonly than the more irregular forms and the sole plate was usually very well marked.

From the foregoing account, it can be seen that it is impossible to attempt to divide the motor end-plates into two types. Real terminaisons-en-grappes have not been found, and although some forms approximate to them, all transitional stages between these and the typical round or oval Rouget end-plate are/

are numerous, and so classification becomes impossible.

Where the myelinated fibre branches before its entrance into the muscle fibre the small branch may be non-myelinated and instead of passing into the end ramifications of the plate, as already described, it may pass to form a small discrete end ramification on the same muscle fibre, either in the same granular sole, and in a small collection of granular substance near the main ending. This is the collateral type of fibre. A number of cases like this have been found. They must be clearly distinguished from the accessory fibres presently to be described. The collateral is simply a branch of the myelinated fibre near its ending, and appears to me to be simply a slight irregularity from the usual type of branching where the branches end all together on the same sole-plate.

A more interesting type of fibre is that seen in fig. 6, which I take to be an ultraterminal fibre. It arises in the terminal ramifications, and passes round the muscle fibre to a completely different level from the end-plate. It is of course impossible to show this in the diagram. In this course upwards, to the right and then to the left, it has upon it two clear nuclei. It ends in a small ramification, the exact position of which it is extremely difficult to determine. It may be situated either on the opposite side of the muscle fibre from the Rouget plate, or on a different muscle fibre lying above.

Another extremely interesting fine non-myelinated fibre is that seen in fig. 7. Its nature, accessory or somatic, that/

that is, a branch of the myelinated fibre from which it passes out, it is impossible to define from this preparation, as its continuity is lost just previous to the myelinated fibre, and its further course, as distinct from that fibre, or as a branch of it, is hidden. The medullated fibre passes upwards to end in a plate, obscured by very deep staining, while the fine non-myelinated fibre passes across a muscle fibre to lie, it appears, between two muscle fibres. It divides into three branches; one passes upwards with slight varicosities and ends after a short course, and the other two, one of which has a nucleus upon it, have varicosities also, appear to join together again, and end in a long loop. The whole termination appears to be outside the muscle fibre, but whether in connective tissue or not it is difficult to state. It may represent one of Kulchitsky's free terminations in the connective tissue of muscle, but of this I am by no means certain.

The Accessory Fibres.

The presence of these fine non-myelinated fibres has already been described in the small nerve branches entering the muscle. In the region of the Rouget plates in the teased specimens of the muscle, a system of fine non-myelinated fibres can be found in the well-stained preparations. In a large number of preparations they were not found, as the staining needs to be extremely good to show them on account of their very delicate structure. I have no doubt that they are the continuations/

continuations of the fine non-myelinated fibres already described in the nerve bundles. They will now be referred to as the accessory fibres, as they appear to me identical with those described by Boeke.

The accessory fibres are found as a rule accompanying the medullated fibres in their course to their Rouget plates. The exact position of these fibres, whether inside or outside the sheath of Henle is difficult to determine. At certain points they show themselves to be definitely outside this sheath, but in a large number of instances they appear to be inside the sheath. This last appearance is in many cases due to the fact that these fibres being very many times smaller in diameter than the medullated fibre and being viewed only from above, will, unless they be at the extreme right or left edge of the sheath appear inside the sheath instead of below or above the sheath. At some point in their course they often take up this extreme lateral position and show themselves to be definitely outside the sheath. In other cases they do not.

They are usually single where found accompanying one medullated fibre, but in some cases more than one can be found in this situation. At intervals along their course they have elongated nuclei which are sometimes seen to be clear and highly refractile, and sometimes more deeply stained. At some points in their course they show a tendency to break up into two or three finer branches only to reunite into the single fibre after a very short distance. This peculiarity is/

is of interest as Boeke figures it in many of his drawings.

Near the end-plate the accessory fibres show various further courses, and according to this further course, they can be divided into four different types. These types are different only in this respect, and cannot be differentiated by any peculiarity of size or structure.

Firstly, the accessory fibres may pass with the medullated fibre into the same sole plate as the medullated fibre and appear to end there (fig. 8). Their exact point and type of termination in this situation cannot in my preparations be determined. They become completely obscured by the very deeply staining ramifications of the myelinated fibre with its knob-like end-points and by the granular sole-plate. It can, however, be stated after thorough examination that they, in these cases, do not pass out from the region of the sole-plate of the Rouget ending. This is not due to the further course of the fibre being interrupted by the teasing, as they can be definitely seen to enter the granular sole-plate. Whether they end, as Perroncito described in the lizard, in a complicated end-net, with branches into the granular substance, or end by joining with the ramifications of the medullated fibre, as Gemelli described, or end in entirely separate small end-nets in the heart of these ramifications, as Boeke described, it is impossible to decide in these preparations. It is unfortunate that their exact termination cannot be seen, but it is only in those cases where the myelinated fibre, its end-ramifications and the granular substance are very deeply stained that/

that the existence and course of the accessory fibres can be followed out at all, and this, of course obscures their final termination in this first type. Fortunately, however, they have other final courses and endings which can be clearly seen and which reveal their nature more conclusively.

Secondly, the accessory fibre may pass away from the medullated fibre just proximal to the latter's formation of the Rouget plate, or pass over or along the side of the Rouget plate to end after a shorter or longer course in an isolated small plate-like ending on the same or on a neighbouring muscle fibre (e.g. fig. 10). In this class the termination of the fibre in a distinctly separate end-apparatus is perfectly clear. Its structure is simply that of the Rouget plate in miniature, with the formation of knob-like expansions in a bed of granular nucleated sole substance. In some cases the heavily stained knob-like appearance is not evident, and a simpler loop-like structure is seen. A large number of instances of this type of termination has not been found in which the identity of the accessory fibre could be followed out for any distance. In a large number of cases the independence of the accessory fibre cannot be shown definitely. It is therefore possible to look upon this larger number of cases as simply collateral fibres of the medullated fibres and not as accessory fibres. That undoubted collateral fibres do exist has already been mentioned, but a number of these doubtful cases seem to me to be more probably accessory fibres than collateral, but this cannot

be/

be proved. The very small diameter of these accessory fibres makes it possible for them to run for long distances just under or above the thick medullated fibres and so their independence is not shown and the appearances suggest that they are branches of the myelinated fibres. As stated above, however, some of the accessory fibres can be traced to their endings over a long course and they are certainly not collateral fibres.

Thirdly, the accessory fibre, associated with the myelinated fibre just before its termination, may pass from the region of the Rouget plate and pass into intimate connection with a neighbouring capillary and run along in intimate contact with the wall of the capillary (fig. 14). The number of instances in which this has been seen and followed for some considerable distance leads to the inevitable conclusion that this is not a chance connection, or a method by which the accessory fibre passes to another muscle fibre or other destination, but that these accessory fibres do in fact supply the capillary wall in the region of the Rouget end-plates.

When a small arteriole is examined and the thicker non-myelinated nerve fibre running along its wall is followed out to the capillaries, which branch out from this arteriole, these undoubted sympathetic fibres are seen to occupy exactly the same relations to the capillary, as regards intimacy, nuclei along their course and thickness, as do the accessory fibres of this type. This observation has been repeatedly made, and

seems/

seems of the greatest importance in interpreting the significance of the accessory fibres. It also demonstrates the probability of the capillaries in muscle being supplied by fibres which reach them by two different routes.

One special case seems of still greater importance, that shown in Fig. 9. In this figure an accessory fibre is seen ending in profile on a muscle fibre, after a short course from a leash of medullated and accessory fibres. The accessory fibre divides up into branches most of which end in darkly staining end-knobs on the muscle fibre forming the small plate, while one of these branches pursues a downward course to be intimately associated and connected with a capillary. This demonstrates conclusively that the accessory fibres which supply the capillaries and those which have small end-plates are of exactly the same origin.

Fourthly, the accessory fibres may pass from the myelinated fibre in the region of the Rouget plate, or from a small nerve bundle directly, and form a plexus by anastomosing with other accessory fibres, from which branches may pass off to have endings of the second or third types. These plexuses are easily demonstrated in the substance of the muscle lying between the muscle fibres.

No ganglion cells have been found in relation to this non-myelinated plexus.

(d) The Neuro-Muscular Spindles.

A large number of these organs have been found and examined. In this muscle no very simple spindles were found, and the majority contained from 2-5 muscle fibres. The spindle muscle fibres were found to be small in diameter and tapering out on either side of the equatorial region for a long distance. In these gold chloride specimens, it has been impossible for me to see the division into daughter fibres inside the capsule. But it is certain that the number of muscle fibres inside the capsule is larger than at either pole outside the capsule. These spindle muscle fibres show as a rule a coarsely marked longitudinal striation, and little collections of deeply stained sarcoplasm round the muscle nuclei. In some few cases, however, these intrafusal muscle fibres do not show the marked longitudinal striation.

The capsule is well shown in these preparations, but does not merit further description here.

The nerve connections of the spindles are of more interest. The endings in the equatorial region are the typical annulo-spiral and flowery terminations described so fully by Ruffini. The first of these is seen to be more extensive and constant than the second. It is formed from a very thick medullated nerve fibre, which often enters the capsule near the equatorial line: of all the intra-muscular nerve fibres it is easily the largest medullated fibre to be seen. While there is often one of these thick fibres, sometimes there are more than one.

The/

The plate-like endings on these muscle fibres are found as a rule outside the capsule towards either extremity of the fibres. In all the spindles which I have examined these endings are formed from medullated fibres. The nerve fibres are not so thick as those forming the annulo-spiral ending, but in every case they are definitely medullated fibres, of about the same calibre as those forming the usual Rouget plates. They enter the spindle often with the very thick medullated fibre and travel outside the equatorial region to form their endings. In other cases they are seen to pass to the spindle from a small leash of motor nerve fibres which is supplying the neighbouring ordinary muscle fibres. In one case, seen in fig. 15, a medullated fibre which passes in from this latter direction, is thicker than is usual for this type of fibre.

The endings are like the small Rouget end-plates seen on the ordinary muscle fibres. They have, I think, a granular sole-plate but it is difficult on account of the coarsely marked longitudinal striation and the little deeply stained masses of sarcoplasm to be absolutely certain of this. They appear to lie hypolemally. The form is often oval or somewhat elongated, while in other cases it is round. In their course along the spindle muscle fibres, these myelinated nerves give off branches which form the end-plates, and it can be quite easily seen, in some cases, that one nerve fibre will end in two places on the same muscle fibre. In fig. 16 the medullated nerve is seen to give off a small branch, which immediately divides into two and these/

these form two plates on the muscle fibre, and the nerve fibre passing down terminates in a third plate all on the same muscle fibre.

A number of the accessory fibres have also been seen passing into the spindle, but the exact mode of termination of all of these very fine non-myelinated fibres cannot in my specimens be seen. Some of the accessory fibres pass in with the thick myelinated nerve and form a very complex network of ramifications inside the capsule, but whether these actually have endings on the spindle muscle fibres or not cannot be determined. Others of the accessory fibres pass in with the nerve fibres to the plates of the spindle muscle fibres and pass with them in a polar direction to end, in thosecases, where continuity is maintained, in relation to capillaries. Fig. 16 shows a case of this type, while Fig. 15 shows a medullated fibre from a motor nerve branch, accompanied by a single accessory fibre which divides up into a number of branches, which have unfortunately been broken.

The following short notes have been made of work done by the same method on the striated muscle of different vertebrates, in an attempt to correlate and verify some of the statements which have been made in relation to the main work on hedgehog muscle.

2. Note on the nerve-endings in striated muscle of the frog.

The two types of muscle fibre in the frog are fairly easily distinguished by size. In my gold chloride specimens, however, they show relatively little difference in their staining, the longitudinal striation being only very slightly more marked in the thin fibres. On the basis of this structural characteristic alone, in my preparations it would be almost impossible to distinguish the two types.

Only two points in relation to their innervation will be dealt with. The innervation of the thin fibres is of great importance in relation to Hunter's work. A very large number of these thin fibres can be found to have endings of the Kuhne type formed from branches of medullated nerves. To be perfectly certain of this somatic innervation I searched for examples, of which fig. 17 is one, in which a medullated nerve fibre divides and supplies both a thick muscle fibre and an adjacent thin muscle fibre; that is the two types of muscle fibre are innervated from the same nerve fibre. Numerous cases of this can be found. No difference then in frog muscle in the innervation of the thin and thick muscle fibres exists, for

at/

at least a large number of the thin fibres. The other point of note in this muscle is the very extensive non-myelinated plexus of nerve fibres which can be seen in it in well stained specimens. Some branches of this plexus are connected with the walls of the capillaries. But I am convinced that the whole plexus is not related to blood vessels alone, although I have as yet been unable to follow out the branches of the plexus to definite endings in the muscle fibres or in the endomysium. Some of these thin non-myelinated fibres have a position similar to the accessory fibres, and are seen running with the myelinated fibre outside the sheath of Henle.

3. Note on the nerve endings in striated muscle of the lizard.

The thin and thick muscle fibres in this muscle show in my few preparations very little difference in their staining. The thin muscle fibre, which has an annulo-spiral ending upon it and is described below has a very well marked cross striation but shows no more marked longitudinal striation than the large fibres.

Accompanying the medullated fibres to the Rouget end-plates, very fine non-myelinated accessory fibres have been found. These are definitely outside the sheath of Henle at certain points. While their full course, especially in relation to the medullated nerve near and at the Rouget end-plate cannot be seen on my specimens, at other points these accessory fibres can be seen to have connections with the fine non-myelinated fibres on the capillary wall.

The thin muscle fibre mentioned above forms in itself a simple spindle. This fibre is lying free in the muscle and is not enclosed in any lamellated capsule. A large medullated nerve fibre forms a very well developed annulo-spiral ending upon it.

4. Note on the nerve endings in the extrinsic eye muscles of the rabbit.

The fibres of these muscles show considerable variations in size and in the degree of longitudinal striation when stained by the gold chloride method, but cannot be classed into two definite types.

The motor end-plates formed from myelinated nerves are found mainly in the posterior part of the muscle, and the sensory terminations mainly in the anterior part of the muscle, although they are found to a certain extent in the part supplied with motor end-plates. The Rouget end-plates are found both on the thick and the thin muscle fibres, and are formed from nerve fibres which retain their myelin right up to the sarcolemma. They show a characteristic branching outside the muscle fibres into as many as five or six short medullated branches which all assist in the formation of the extensive terminal ramifications in a well marked granular sole-plate.

The sensory fibres give off many fine non-myelinated fibres at the nodes of Ranvier and end by running for very long distances as non-myelinated fibres. These non-myelinated fibres/

fibres end in small collections of darkly stained knobs connected together by very fine branches. These may become more extensive and many may be found on one fibre. They show no sign of a granular sole plate.

I have been unable to find accessory fibres running with the motor fibres and on account of the large number of non-myelinated fibres, which are branches of the sensory medullated fibres, the nature of thin non-myelinated fibres, which show no definite connections in their short visible uninterrupted course, is extremely difficult to determine in this muscle.

5. Note on the nerve endings in human pectoral muscle.

I have to thank Dr J. Scoular Buchanan for the opportunity of obtaining small pieces of pectoral muscle in a perfectly fresh condition from the operating theatre.

In this muscle, a great uniformity in the size of the muscle fibres is found, and very little difference between individual fibres by this method of staining can be observed.

The nerve fibres, to the motor end-plates, are thinner than those in the hedgehog, and show in a very marked degree, the loss of their myelin, while still at some distance from their termination. This at once makes the question of non-myelinated fibres, which are of other nature than these de-myelinated somatic fibres, much more difficult than in the hedgehog, as non-myelinated fibres which do not show their origin in a preparation may either be fibres which are non-myelinated throughout their whole course, or simply fibres which/

which have lost their myelin. The Rouget end-plate seen in this muscle, is extremely simple in form, as the terminal ramifications are short and thick and do not branch much. In spite of the difficulty of non-myelinated fibres, I have, I think, seen in this muscle extremely fine accessory fibres accompanying a medullated nerve fibre. Their endings I have been unable to find as yet.

One point is of interest in relation to the muscle spindles, which are very complex. A very large number of medullated nerve fibres is found entering the capsule, the largest of which form the annulo-spiral ending and the smaller passing out to the extremities of the spindle form little end-plates on the spindle muscle fibres. These fibres, forming the end-plates are definitely myelinated.

PART IV.

DISCUSSION.

-----oOo-----

The question of the pluri-segmental motor innervation of the individual muscle fibres does not require any further discussion. The existence of such a condition in this muscle of the hedgehog has already been described and the points in which it differs from the demonstration given by Agduhr for limb muscles have been noted. What physiological significance can be placed upon it, it is difficult as yet to suggest. It may, however, like the sympathetic innervation, be related to the tonic activities of the striated muscle. For example, it may in some way have to do with the inhibition of tone in reciprocal innervation.

The existence of two types of muscle fibre throughout vertebrate muscle, is a much more difficult question, and one which is of special significance in view of Hunter's hypothesis, that the two types receive entirely different innervations and perform entirely different functions. If such a condition as Hunter suggested, be present, then it seems to me a matter of great importance to determine whether the two types remain entirely discrete, or whether the two types are the extreme conditions and all or many intermediate forms exist between them. If the former condition holds good, then it is easily conceived how such an innervation, one type by somatic fibres and/

and another type by sympathetic fibres, could exist. But if the latter condition be present then it seems to me extremely difficult to know how such an innervation could be postulated for the intermediate muscle fibres between the two extreme conditions. Such a muscle fibre would have a function intermediate between the functions of the extreme fibres. How such fibres would be innervated it is difficult to imagine, since Hunter denied the possibility of a double innervation of the individual fibre.

Now this second condition does, I think, occur in vertebrate muscle. All stages between the clear sarcoplasm-poor fibre and the dull sarcoplasm-rich fibre, are found in those muscles in which any variation is found. Such a condition is found in the panniculus carnosus. Grützner's figures of the guinea pig muscle also suggest this, and Cilimbaris stated it definitely for the extrinsic muscles of the eye. Even Kulchitsky's figures on which much of Hunter's hypothesis rests do not show a clear distinction into two types of muscle fibre, and it is significant that Kulchitsky himself did not attempt to make this distinction and made no reference to the size or structure of the muscle fibres on which his type I and type II endings terminated. In the frog muscle, where the differences are easily demonstrated, in a fresh preparation examined in saline it is not possible always in the largest of the thin fibres and smaller of thick fibres to say to which type they belong. In connection with another investigation I made cross sections/

sections of the sartorius muscle of the frog and measured the cross-sectional area of each individual muscle fibre under the same magnification. It is interesting that nearly all the thin fibres are found on the outer surface of the muscle and all the broad fibres beneath this outer layer. Over a number of such experiments it is not possible, in a graph of the frequency of fibres in small groups of nearly similar areas, to state definitely that there are two types of fibre. On account of size therefore in this muscle, no definite separation of two types can be made, as a gradual transition is found from the smallest to the largest fibres.

In view of these histological considerations it seems to me unlikely that the two <u>distinct</u> types of fibre do exist in vertebrate muscle, and on this account that the condition of double innervation as regards a whole muscle only, and a single innervation of the individual fibres, is improbable. More definite evidence of this last statement has been found. My preparations do demonstrate that the fibres showing marked longitudinal striation and more sarcoplasm are innervated by myelinated nerves, and show endings like those of the mass of fibres which do not show this well-marked longitudinal striation. I do not think that in this muscle any muscle fibres are to be found which do not have a medullated nerve fibre ending upon them. According to Hunter's theory the thin muscle fibres of the frog should only be supplied by the sympathetic fibre and as this point is easily established in a number of preparations of/

of frog muscles by the gold chloride method, I have made a short note on this point. They show conclusively, that neglecting for the moment the sympathetic endings, the thin muscle fibres do have medullated nerve fibres ending upon them in small typical end-bushes of Kuhne. If a double innervation of frog's muscle exists, then on the basis of this note it must be a double innervation of each individual fibre and not only of the muscle as a whole.

From the functional point of view, in frog's muscle it is extremely difficult to maintain Hunter's theory. The two types of frog muscle fibre have long been known to show two different types of contraction, the large clear fibres giving a quick response, and the thin dull fibres a slow sustained response. Hunter maintained however that the thin fibres were concerned with plastic tonus and were innervated solely by sympathetic fibres. Now since stimulation of the sympathetic, excluding the work of Orbeli to be described later, has always given negative results and since the two types of contraction have been obtained by stimulation of motor nerves, it may, I think, be presumed that these two types of contraction have been due to stimulation of somatic fibres. The fact that these two types of contraction have been obtained seems to me to furnish further evidence that the thin fibres have been activated by stimulation of the somatic fibres and must therefore be innervated by the medullated fibres, whether they have an additional nerve suppky through the sympathetic or not. From experiments/

experiments which are now being conducted I think it may be said definitely that evidence of the two types of muscular action in a frog's gastrocnemius may be seen after removal of the sympathetic chain on one side and stimulation of the 9th and 10th spinal roots above the junction of their respective Rami Communicantes, thus excluding any possibility of one type of muscle fibre being innervated solely by the sympathetic.

The work of Kulchitsky is certainly in favour of one set of muscle fibres being innervated by the sympathetic only. It must, however, be remembered that Kulchitsky's work was done on snake muscle, which may differ from mammalian muscle. No degeneration experiments have yet been undertaken on snake muscle to prove the sympathetic nature of the fibres and endings of his Type II.

All the other histological evidence, excluding the unpublished work of Hunter and Latham referred to above, is in favour of the double innervation, somatic and sympathetic of individual muscle fibres, and my own work is certainly in favour of this theory.

The work of Kahn on the clasping muscles of the frog adds further evidence in favour of the single muscle fibre having a double innervation. In the season of increased tone, if this function were performed by one type of fibre one would expect an increase in their number, that is, an increase in the number of thin granular fibres. On the contrary he found that/

that the number of fibres was not increased but that the sarcoplasm of the broad clear fibres was increased in amount.

The nature of the fine non-myelinated fibres which I have called accessory fibres, must now be considered. It is interesting to recall the six points, made by Boeke, (9) in which he compared his accessory fibres stained by silver methods with the fine fibres described by Perroncito and Gemelli. This is necessary to see if the term 'accessory' may be fitly applied to the non-myelinated fibres I have described in my gold chloride preparations.

(1) The fibres described here are like Boeke's in that they are often single when accompanying a medullated fibre. Fig. 15 is the only noteworthy exception to this and shows a single fibre breaking up into a number of branches while accompanying a medullated fibre to a spindle muscle fibre.

(2) The fibres are shown to be definitely outside the sheath of Henle at certain points, and are like Boeke's in this respect.

(3) Very small end-plates distant from the motor end-plates are formed from the fibres as Boeke showed.

(4) The great uniformity of Boeke's accessory end-loops cannot be borne out by my preparations.

(5) The granular sole-plates of the accessory end-plates of these fibres has been described, like those of Boeke.

(6) No degeneration experiments have been made. I think it may be concluded, in spite of (4), that these fibres are identical with those of Boeke and I have used the term *'accessory' with this meaning.

The accessory fibres, which pass into the same granular sole plate, as does the medullated fibre, cannot be traced out completely after they enter the plate and so further comparison with the fibres described by Perroncito and Gemelli is not possible.

Boeke, on the basis mainly of his degeneration experiments, where the accessory fibres were found intact after the section and degeneration of all the somatic fibres, concluded that the accessory fibres were of sympathetic origin and maintained that the individual muscle fibre received normally a somatic and sympathetic innervation.

I have made no such degeneration experiments, but I think that some conclusions as to the nature of these fibres may be drawn from my preparations. That they are not branches of the medullated fibres, at least in the muscle, is I think certain, from my finding, in the intra-muscular nerve bundles and nerves * about to enter the muscle, of a large number of accessory fibres. I hold that they continue down with the medullated fibres and are the same fibres which are seen in the teased specimens of the muscle. The finding of these fine non-myelinated fibres in the muscular nerve is in harmony with the results of Sherrington and Ranson, but not with those of Langley. My only criticism of Langley's work, as I have suggested already, is that I am not/

not convinced that the osmic acid method would show in cross section or in teased preparations, such fine fibres, as it is possible to demonstrate with the gold chloride method, especially if these fine fibres are distributed throughout the whole nerve and not confined to one part, where they could be seen "en-mass."

The plexus formation of the accessory fibres is of importance in attempting to come to conclusions as to their nature. This plexus formation is totally unlike the somatic fibres and is characteristic of a nervous system of a lower type, the vegetative or sympathetic nervous system as it is seen in vertebrates.

The different terminations of branches of this accessory nerve plexus also throw light on the nature of the fibres forming it. I have been unable to find any difference between the accessory fibres which pass to the capillary wall and those which end on the striated muscle fibre, and am of opinion that the two types of fibre have exactly the same origin. This seems to me to be demonstrated by such a fortunate specimen as fig. 9 where one branch of the same fibre supplies a capillary wall and the other a muscle fibre. I have also been unable to find any difference between the accessory fibres and those definitely sympathetic non-myelinated fibres which pass down to the capillaries from the arterioles.

For these reasons, then, I believe, that the accessory fibres which I have demonstrated and described, belong to the/

the sympathetic nervous system.

Before considering what function these sympathetic fibres may perform, two points, in consideration of their extensive connections with the capillary wall, are of interest. Besides the usual route of nerve supply to the capillary wall, that is, simply an extension of the plexus of pale nerve fibres found round the arteriole, I have described a second route, in muscle, where the non-medullated fibres pass direct to the capillary wall from the medullated nerve bundles near the Rouget plate endings.

Whether these two routes are of physiological significance or not, it is difficult to say, but it is not improbable that they may represent the different routes of supply of the two sets of vasomotor nerves, the vaso-constrictors and vaso-dilator fibres. If such be the case, it seems more likely that those fibres which pass directly from the medullated fibre bundles, are of vaso-dilator function, and come into action with the accompanying spinal motor nerve fibres. While these suggestions are interesting, they are entirely insupported by any evidence at the moment.

The other point of interest, in relation to the extensive connections of the accessory system with the capillaries, is that whatever function it may have in the striated muscle fibre, this function must be intimately associated with the vasomotor mechanism. It seems to me difficult to believe that those/

those fibres which pass to the striated muscle could be set in action without the fibres to the capillaries being involved at the same time, and therefore, variations in the action of the fibres to the striated muscle will be accompanied by changes in the vasomotor mechanism.

Two possible functions have been suggested for the sympathetic innervation of striated muscle; it may fulfil either a trophic or a tonic function. In 1920 Adrian (1) in a short review of this subject stated naively - "At present we have only one reason for supposing they have any function at all and that is the fact that they exist." This followed upon a review of the very contradictory and unconclusive experimental results, which had been recorded by a number of workers.

It is worth while to note here, that we are dealing with a nerve net, and that we cannot expect to get such a clear picture from our experimental work, as we have grown to expect in relation to our work on the synaptic system. In experiments in which the sympathetic rami have been cut, or the ganglia removed, it is very difficult to know just how much degeneration will take place in a nerve net of this kind. It seems certain that the degeneration will occur very much more slowly than in the synaptic system, if indeed much degeneration does occur. It is probable that the nerve net may be kept intact at least in part by connections which pass to it by way of the blood vessels. This may account for a number of the failures to get variations/

variations in the tone of the muscle after these operations, if indeed the sympathetic innervation is of tonic function.

Orbeli (1923)(42) alone on the basis of some work by Ginezinsky, has recently put forward evidence that stimulation of the sympathetic has any effect on striated muscle. In the frog he found that sympathetic stimulation had no added effect during stimulation of the somatic roots to the gastrocnemius while the muscle was responding well, but that after the muscle began to show signs of fatigue, sympathetic stimulation would give a definite increase in the response obtained on stimulation of these somatic roots. After a considerable latency, the muscle showed increased force, amplitude and rapidity of development of contraction, amd the after-effect was prolonged. He therefore thought the action of the sympathetic on skeletal muscle was analogous to the action of the sympathetic on cardiac muscle. As all the vital activities of skeletal muscle are stimulated by the sympathetic, he said the influence of the sympathetic corresponded to that of Pavlov's trophic nerves.

In the experiments of Bottazzi (15), already described, this idea of a trophic function of these sympathetic fibres was not borne out, as after removal of the somatic influence to the diaphragm the muscle degenerated. It is difficult to judge the value of experiments of this nature or similar nature, for while the sympathetic may have a trophic function it does not seem to me reasonable to suppose that it will be of such strength/

strength as to keep a completely inactive muscle in normal condition.

In this connection, Sherrington (1894)(55) described the muscular nerves in an amyelous foetus, which is interesting from this point of view and also appears to have some relation to a point to be considered later, the persistence of the normal appearance in the spindle muscle fibres after complete denervation. In this foetus he found that all the muscular nerves were present although there was not a single motor spinal nerve root present in any segment of the cord. All the somatic fibres present in the so-called motor nerves to muscle were sensory. It is of great interest that the striated muscles were normally developed. The trophic influence of the anterior horns must therefore, Sherrington concluded, be in abeyance during foetal life. He also suggested that the muscular degeneration associated in later life with the section of muscular nerves was due less to a lack of normal trophic influence than to the enforced inactivity of muscles, which are accustomed to be active. It is also worthy of note that the sympathetic system was found fully developed and normal.

It may, I think, therefore be considered quite unlikely that, if the sympathetic has a trophic influence on striated muscle, that it will be able to exert this influence if the muscle is completely inactive. From the physiology of the peripheral nerve nets in lower animals (Parker 1920)(44) it seems/

seems clear that the stimulus to which a nerve net is most adapted is the mechanical stimulus. It is therefore most probable that whatever the function of the sympathetic in striated muscle, trophic or tonic, it will be influenced mainly by the mechanical stimulus of the movements of the muscle fibres brought about by the somatic nerve fibres. It is, for this reason, I think, that stimulation of the sympathetic alone has so consistently given negative results.

Whether the sympathetic subserves a trophic function in the striated muscle or not, must in view of these considerations be left an open question at present.

The question of the tonic function of the sympathetic in striated muscle is also extremely difficult. It is necessary to consider some of the work that has been put forward to explain its exact mode of action.

Bottazzi (1897)(14) put forward a theory the simplicity of which is attractive. He held that the sarcoplasm of the fibre was responsible for the tone, while the striped apparatus, or sarcostyles were responsible for the rapid movements. This theory was based on observations of the rhythmic changes of tone in the heart of the tortoise, where a very large amount of sarcoplasm was found in one layer of the cardiac muscle cells and on the supposition that the more specialised movement would be performed by the specialised apparatus - the sarcostyles, and the primitive type of slow movement or tonic contraction would be performed by the unspecialised more primitive sarcoplasm.

This/

This theory is supported by the two different forms of contraction seen in the different types of frog muscle, the fibre showing more sarcoplasm having a longer period of contraction. But the difficulty in this case is that there seems to be some confusion, between the normal tone or tonus of the muscle and a prolonged contraction of these thin fibres - what is sometimes described as a "tonic" contraction. It must be proved that the two mechanisms are identical before this piece of evidence really supports Bottazzi's theory.

Kahn's work, already referred to, seems good evidence in favour of Bottazzi's theory. He used a natural seasonal increase in tone and found an anatomical difference in the broad fibres which showed a marked increase of their sarcoplasm. He did not state that the increased amount of this substance was directly responsible for the increase in tone, but that it was definitely associated with it, was clear.

Bayliss (1924)(5) stated "whether the one function is performed by the sarcoplasm, as held by Bottazzi and the other by the fibrils remains undecided. It is not easy to understand how two fibres of different function could coalesce with retention by the combined cell of both kinds of innervation although the case of the salivary glands suggests the possibility." It appears to me that there has been no coalescence of the two fibres of different function, as both types of fibre show both sarcoplasm and sarcostyles, though in different degrees, and rather it is a case of the sarcostyles developing to different degrees from the more primitive sarcoplasm of the/

the individual fibre.

Mosso (1904)(41) stated that he was unable to accept Bottazzi's theory because he did not find that the oscillations of tone were most marked in those fibres which showed a large amount of sarcoplasm, nor were they least marked where the sarcoplasm was only present in a very small degree. He therefore proposed a new theory, based on Perroncito's work, that the tone of the muscle was related to the double innervation of the single muscle fibre by somatic and sympathetic fibres. He concluded this paper thus "There is probably in the structure of the muscle, one contractile substance, which reacts to two excitations, of which the one comes from the myelinated fibres and the other from the fibres of the sympathetic."

On the firmer histological basis of Boeke's work, de Boer (1915)(13) put forward the view that the sarcoplasm innervated by the sympathetic fibres had a tonic function, while the sarcostyles, innervated by the somatic fibres, were responsible for the rapid movements. It is of interest here that on both the nerve endings, somatic and sympathetic, Boeke described his periterminal network, which he thought was connected to the anistropous substance of the sarcostyles. He does not state definitely whether the periterminal network of the accessory fibre was also connected with the sarcostyles; if it, like the medullated fibre's network, is connected with the sarcostyles, then De Boer's theory cannot be accepted.

Langelaan (1915)(38) and (1922)(39) carried De Boer's theory/

theory further. He distinguished two different components of muscle tone, the plastic and the contractile factors. The plasticity of the muscle he described as that property by which any change in the length of the muscle caused by external forces was rendered permanent to some degree. The greater the degree of plasticity, the greater is the permanent deformation. It is evident that such a permanent deformation will not be accompanied by a continued expenditure of energy nor by electrical phenomena, or at least the expenditure of energy will be extremely small. This plasticity he thought was intimately associated with the trophic state of the muscle and the degree of plasticity may prove to be a measure of the trophic state. Plastic tonus is a reflex tonus, which shows itself after movements and not during them. It is concerned with attitude and posture. Contractile tonus on the other hand, is the slight degree of contraction in the muscle, and was, he maintained, the component which Brondgeest in his classic experiment demonstrated. This, unlike the plastic tonus, is not a state of equilibrium and is accompanied by an expenditure of free energy and by rhythmic electrical phenomena. It is associated with the production of heat in muscle.

From Boeke's demonstration of the sympathetic fibres in muscle, Langelaan put forward the theory, that the plastic component of muscle tonus is the property of the sarcoplasm and is under the control of the sympathetic system and the contractile component of muscle tonus is the property of the striped/

striped apparatus of the fibre and is under the control of the cerebro-spinal system.

This hypothesis of Langelaan includes and extends the theories of both Bottazzi and De Boer, and in many ways clears up the conflicting results of other workers. Mosso's criticism of Bottazzi is now of little value as he was evidently referring to contractile tonus and not to plastic tonus. That the sympathetic alone is concerned with muscle tone, as De Boer held, may be abandoned in view of much of the experimental work and of Langelaan's more complete exposition. The negative results of many of the workers, after extirpation of the sympathetic, or section of the Rami communicantes, is explained by the fact that they were attempting to get changes in the contractile component and not in the plastic component of the muscle tonus. It also fits in with the view that the nerve net is more adapted to a mechanical stimulus. While there are points in this hypothesis which are not yet clear, it appears to me to fit the known facts best. Langelaan's hypothesis is also correlated to Orbeli's work in that Langelaan suggested that the degree of plasticity may be an index of the trophic state of the muscle.

Hunter (1925)(33) is in agreement with Langelaan in the distinction of the two components of muscle tonus, but differs in this that he, on the basis of the idea of Grützner (1887)(30) of the "inner support" of the red fibres in a mixed muscle, attributed the two components to different muscle fibres. The reasons/

reasons for not accepting this hypothesis have already been given, - the unproved existence of the two distinct types of muscle fibre in all vertebrate striped muscle and the certain innervation of the sarcoplasm-rich fibres in some muscle by medullated fibres. Hunter and Royle however did clearly demonstrate the loss of plasticity in the muscle after removal of the sympathetic influence. They found that the longer the period after sympathetic ramisectectomy the clearer was this demonstration, which may have some relation to the length of time required to get marked degeneration in the nerve net of the non-myelinated fibres in the muscle.

The action of adrenalin has as yet given little aid in the solution of this problem. It cannot be expected in view of Langelaan's hypothesis, that it should show any direct action on muscle. All that might be expected is that it would increase the plasticity of the muscle, after stimulation. There is some slight evidence in favour of this.

The reflex origin of muscle tonus is undoubted and it must I think be in some way related to the neuro-muscular spindles. The exact function of these structures has not yet been ascertained; it has been suggested that they are related to the proprioceptive sense. I think it may be taken that they are supplied with three types of nerve fibre, the main sensory fibres, the motor fibres and the accessory fibres, and any attempt to interpret their function must take cognisance of these three sets of fibres. My results do not confirm Kulchitsky's/

Kulchitsky's observations in the snake muscle, where he found the end-plate of the spindle muscle fibres to be formed from sympathetic fibres. In the hedgehog and in man I have found the end-plates formed from medullated fibres. Sherrington (55) revealed the fact that complete denervation does not cause degeneration of the spindle muscle fibres. In view of his suggestion following his description of the amyelous foetus, that it was only after the muscle had been active, that is after birth, that the section of the muscular nerve caused degeneration, and that this was probably due to inactivity, it seems possible that the spindle muscle fibres have never been accustomed to movements like those of the ordinary muscle fibres and so they do not degenerate upon section of the nerve. Sherrington (55) was of opinion that these muscle fibres responded to a mechanical stimulus only, as he maintained they had no motor nerve fibres. This mechanical stimulus was the movement of the mass of fibres surrounding them. I do, however, believe that they have motor fibres and that they respond to nervous, not mechanical, stimuli, which may be of reflex origin. In those muscles which do not show any marked variation on their ordinary muscle fibres, the spindle muscle fibres are certainly as a rule clearly distinct from the ordinary muscle fibres, and show as thinner, fibres which are richer in sarcoplasm. These I think represent the thin fibres of the frog muscles, where the thin spindle muscle fibre lies free in the mass of the muscle and have little or no capsule.

Sherrington (55) described an interesting experiment which he thought threw some light on the action of these spindle muscle fibres. After section of the ventral roots for a period of 42 days, the muscles retained some degree of response to faradic stimulation. Fairly strong stimulations applied to the nerve of such a muscle caused no muscular response, but very strong stimulation resulted in a peculiar contraction - a slow feeble steady contraction lasting for 5 seconds after stimulation ceased. Crushing of the nerve peripheral to the point of stimulation stopped the phenomenon, and it was not obtained after excising the spiral ganglion. It had nothing to do with regeneration of the nerve fibres. He added "it is tempting to relate it to the intrafusal muscle fibres." These muscle fibres would certainly have normal appearances and it may be that this contraction was a reflex through the spiral ganglion and passing out by the sympathetic fibres. It is difficult to see how this contraction, if it is due to the contraction of the spindle muscle fibres, could be set up by a mechanical stimulus as the ordinary muscle fibres would be degenerated and could not react sufficiently to give a mechanical stimulus to the spindle.

The annulo-spiral ending of the spindle seems specially adapted to receive impressions of varying cross sectional area of these spindle muscle fibres, such as would result from a wave of contraction in these fibres. The afferent impulses set up in this way, may be either of proprioceptive nature or be the afferent/

afferent impulses of a tonic reflex arc. The thick capsule round the region of the annulo-spiral endings and the peri-axial lymph space seem to be special adaptations to ensure that the sensory fibres will only be acted upon by the action of the spindle muscle fibres and not by the pressure of the contraction of the ordinary muscle fibres.

I wish to acknowledge my indebtedness to Professor Camillo Golgi, in whose laboratory I learned the method used in this research and who by his kind and helpful attention made it possible for me to attempt this work. I have also to thank Professor D. Nöel Paton and the staff of the Institute of Physiology for their unceasing interest which has been an inspiration to me through the whole period of my work.

PART V.

Conclusions.

1. The muscle fibres of the panniculus carnosus of the hedgehog show varying degrees of longitudinal striation and varying contents of sarcoplasm, but cannot be divided into two definite classes.

2. The nerves entering the muscle contain a large number of very fine non-myelinated nerve fibres.

3. The motor end-plates show great variations in size, form, and shape but cannot be classed into two definite types.

4. The muscle fibres are individually pluri-segmentally innervated, in a slightly different manner from that which Agduhr demonstrated in limb muscles.

5. All the muscle fibres whether rich or poor in sarcoplasm appear to be innervated in exactly the same way. It is certain that the sarcoplasm rich fibres are innervated by medullated nerves.

6. The presence of Boeke's accessory fibres and endings can be confirmed. They appear to be a system of fibres completely separate from the somatic fibres and are, I think, of sympathetic origin.

7. The accessory fibres have been seen to end in some cases on a muscle fibre which is also supplied by a medullated fibre.

8. The accessory fibres show connections with the capillary wall, and two different routes of nerve supply to the capillaries are/

are demonstrated, one by extension from the arteriole and the other direct by way of the motor nerve fibres.

9. The plate endings on the spindle muscle fibres are formed from definitely medullated nerve fibres.

10. In frog muscle, the thin and thick muscle fibres are both supplied by medullated motor nerve fibres, in many cases one medullated fibre supplies both types of muscle fibre.

11. Extremely little variation exists in the size and structure of the muscle fibres of human pectoral muscles.

12. Accessory fibres are present in frog, lizard, and human muscle.

13. The plate-like endings on the human muscle spindles are formed from medullated fibres.

PART VI.

Description of Figures.

These drawings I have made with a camera lucida, as photographs are of very little value on account of the varying levels of the structures in teased preparations. (The figures in the drawings refer only to the number of the slide).

Figs.1-16 are from the panniculus carnosus of hedgehog.

Fig. 1. A dissociated muscle fibre of the panniculus carnosus, showing two motor end-plates on the same muscle fibre a long distance apart. An example of the pluri-segmental innervation of the individual fibre.

Fig. 2. A small intra-muscular nerve bundle showing the presence of accessory fibres at some distance from the terminations of the medullated fibres of this bundle.

Fig. 3. Three motor end-plates of different size. The two small plates shown in profile receive fibres which are non-medullated near the plates but are medullated further up the small nerve bundle.

Fig. 4. Two motor end-plates of different size formed from branches of same medullated fibre. Small plate in profile.

Fig. 5. Three motor end-plates, the lowest of which shows a fine non-myelinated fibre arising just proximal to the entrance of its nerve fibre into the muscle. The nature of this fibre is difficult to determine; its accessory nature may be hidden, or it may be simply a collateral.

Fig. 6. A motor end-plate, showing an ultraterminal fibre which passes out from the terminal ramifications to the right, passes downwards and rises to reach a different level and then passes to the left. It forms a small end arborisation, which lies either on the opposite side of the small muscle fibre or on another fibre.

Fig. 7. A very fine non-myelinated fibre whose nature is not definitely seen. The ending probably lies between the muscle fibres. The medullated fibre passes upwards to form a motor plate which is obscured by very deep staining.

Fig. 8. Two medullated fibres passing upwards to form motor end-plates. Between them and lying outside the sheath of Henle, which is shaded in pencil, lies an accessory fibre, which ends by passing to the motor plate on the right. (Accessory fibre of 1st type). Capillaries - stippled.

Fig. 9. An accessory fibre of the 2nd type which passes across from the nerve bundle on the right to form an accessory plate, seen in profile on a large muscle fibre. The thickening just as it leaves the nerve bundle is a deeply stained nucleus. The accessory fibre divides into very fine branches which form the ending. One of the fine branches passes downwards to become intimately connected with the wall of a capillary. Capillaries - stippled. (The horizontal capillary passes over the end-plate and is not drawn in full to give a clearer picture).

Fig. 10. Plexus of accessory fibres. One of these ends in a small plate at the foot of the drawing. This fibre lies at a different level from the one which it crosses over.

Fig. 11. Plexus of accesssry fibres. The exact termination of many of the plexus branches is not clear, but one can be seen forming an end-plate on a narrow muscle fibre, which on being traced out is found to be the tapering end of a large fibre. Another branch may be seen passing to a capillary. Capillaries - stippled.

Fig. 12. Plexus of accessory fibres.

Fig. 13. An accessory fibre passes down with a thick medullated fibre. It appears to branch and some of the branches appear to end round or in the motor plates, while others pass definitely to capillary walls. Capillaries - purple.

Fig. 14. An accessory fibre of the 3rd type which passes to a capillary wall. The course of the accessory fibre over the plate is obscured. It runs close to but does not I think supply the capillary on the left, but it certainly supplies the capillary on the right. Capillaries - stippled.

Fig. 15. A thick medullated fibre from a motor nerve branch, which passes to form a plate on a spindle muscle fibre. It is accompanied by an accessory fibre which divides up into a large number of fibres which branch and reunite among themselves. These accessory fibres cannot unfortunately be followed in this preparation; some of them are torn. Motor end-plates below in pencil.

Fig. 16. One end of a spindle muscle fibre. The cross striping is diagrammatic. A medullated fibre is seen to end in three plates on the muscle fibre. Accompanying it is an accessory fibre of the third type passing to end in relation with capillaries. Capillaries - stippled.

Fig. 17. Frog muscle. One thick muscle fibre and two very thin muscle fibres supplied by one medullated nerve fibre. All three endings are typical Kuhne end-bushes, those of the smaller muscle fibres being in no way different, except that they are smaller.

REFERENCES.

-----oOo-----

(1) ADRIAN, 1920. Med. Science. II. No. 5, p. 454.

(2) AGDUHR, 1916. Anat, Anz. 49. p. 1.

(3) AGDUHR, 1919. Anat. Anz. 52. p. 273.

(4) AGDUHR, 1918. Proc. Kon. Akad.v.Wet.t. Amsterdam, XXI. p. 1231.

(5) BAYLISS, 1924. Principles of General Physiology. 4th Edit. p. 546.

(6) BOEKE, 1909. Verslag. Kon. Akad. v. Wet. t. Amsterdam, April 1909.

(7) BOEKE, 1909. Anat. Anz. 35. p. 193.

(8) BOEKE, 1910. Anat. Anz. 35. p. 481.

(9) BOEKE, 1913. Anat. Anz. 44. p. 343.

(10) BOEKE, 1916-17. Verh. d. Kon. Akad. v. Wet. Amsterdam, 18. p. 91. and 19. p. 1.

(11) BOEKE, 1921. Brain. 44. p. 1.

(12) BOEKE and DE BARENNE, 1919. Proc. Kon. Akad. v. Wet. t. Amsterdam. XXI. 1227.

(13) DE BOER, 1915. Ztschr. f. Biol. 65. p. 239.

(14) BOTTAZZI, 1897. Jour. Phys. 21. p. 1.

(15) BOTTAZZI, 1923. Quart. Jour. Exper. Phys. Supplementary Vol. p. 66.

(16) BOTEZAT, 1906. Zeitsch. f. Wiss. Zool. 84. p. 228.

(17) BOTEZAT, 1910. Anat. Anz. 35. p. 396.

(18) BREMER, 1882. Arch. f. Mikr. Anat. 21. p. 169.

(19) CECCHERELLI, 1902. Monitore. Zool. Ital. Ann. XIII n. 9.

(20) CILIMBARIS. 1910. Arch. f. Mikr. Anat. 75. p. 692.

(21) DART, 1924. Jour. Comp. Neur. 36. p. 441.

(22) DOGIEL, 1902. Arch. f. Mikr. Anat. 59. p. 1.

(23) FUSARI, 1903. Arch. Ital. de Biol. 39. p. 473.

(24) FUSARI, 1907. Arch. Ital. de Biol. 48. p. 472.

(25) GEMELLI, 1906. Monitore. Zool. Ital. Ann. XVII.

(26) GOLGI, 1903. Opera Omnia 1. p . 211.

(27) GOLGI, 1919-20. Dispense di Istologia. Parte Generale. Anno. 1919-20. p. 188.

(28) GRABOWER, 1902. Arch. f. Mikr. Anat. 60. p. 1.

(29) GRUTZNER, 1884. Rec. Zool. Suisse. I. p. 665.

(30) GRUTZNER, 1887. Arch. f.d.g. Physiol. 41. p. 280.

(31) HUBER, 1899. Anat. Anz. 15. p. 335.

(32) HUBER and DE WITT, 1897. Jour. Comp. Neur. 7. No. 3 and 4.

* (33) HUNTER, 1925. B.M.J. Jan. 31. p. 197.

(34) KAHN, 1924. Arch. f.d.g. Physiol. 205. p. 381.

(35) KULCHITSKY, 1924. Jour. Anat. LVIII. p. 152.

(36) KULCHITSKY, 1924. Jour. Anat. LIX. p. 1.

(37) KUNTZ and KORPER, 1924. Proc. Exp. Biol. and Med. XXII. p. 23

(38) LANGELAAN, 1915. Brain. 38. p. 235.

(39) LANGELAAN, 1922. Brain. 45. p. 434.

(40) LANGLEY, 1922. Jour. Phys. LVI. p. 382.

(41) MOSSO, 1904. Arch. Ital. de Biol. 41. p. 183.

(42) ORBELI, 1923. J. Petrograd. Med. Inst. 6. p. 8.

Reviewed by -

(43) WALSHE, 1924. Med. Science. X. No. 6. p. 486.

(44) PARKER, 1920. The Elementary Nervous System.

---

* This reference to the lectures which Hunter had prepared but unfortunately did not deliver is given in preference to the published original papers as more details are given of the histological basis of his work.

(45) PEMBREY, 1903. Jour. Phys. XXIX. p. 195.

(46) PERRONCITO, 1901. Arch. Ital. de Biol. 36. p. 245.

(47) PERRONCITO, 1902. Arch. Ital. de Biol. 38. p. 393.

(48) RANSON, 1911, Amer. Jour. Anat. 12. p. 67.

(49) RANVIER, 1887. Compt. Rend. CIV. p. 79 and 1888, CVII. p. 971.

(50) RETZIUS, 1892. Biologische Untersuchungen. Neue Folge. Bd. III. p. 48.

(51) FFRANGCON ROBERTS, 1916. Brain. 39. p. 297.

(52) RUFFINI, 1898-99. Jour. Phys. XXIII. p. 190.

(53) RUFFINI, 1900. Riv. di patol. nerv. e. ment. V. fasc. X.

(54) SCHAFER, 1912. Quain's Anatomy. Vol. II. Part I. p. 190.

(55) SHERRINGTON, 1894. Jour. Phys. XVII. p. 211.

(56) SUTTON, 1915. Amer. Jour. Anat. 18. p. 117.

(57) TSCHIRIEW, 1879. Arch. de Phys. Nerv. et Path. VI.

(58) WILSON, 1921. Brain 44. p. 237.

I

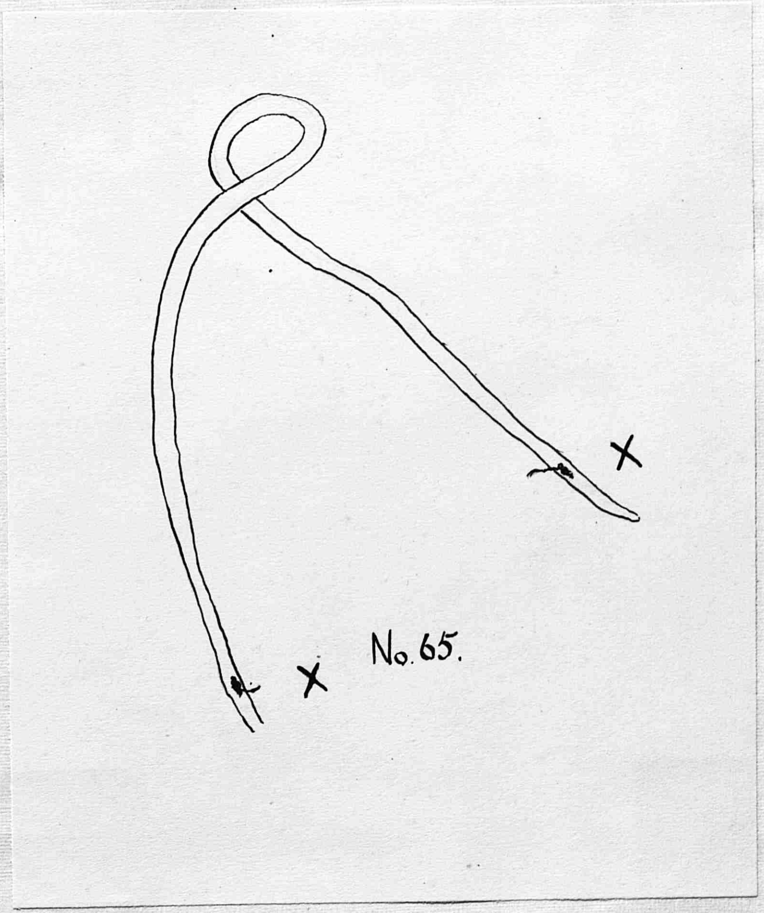


FIG. 1.

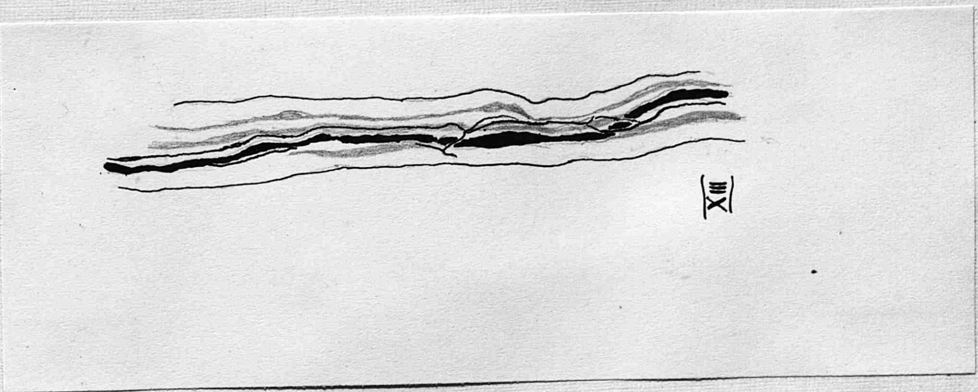


FIG. 2.

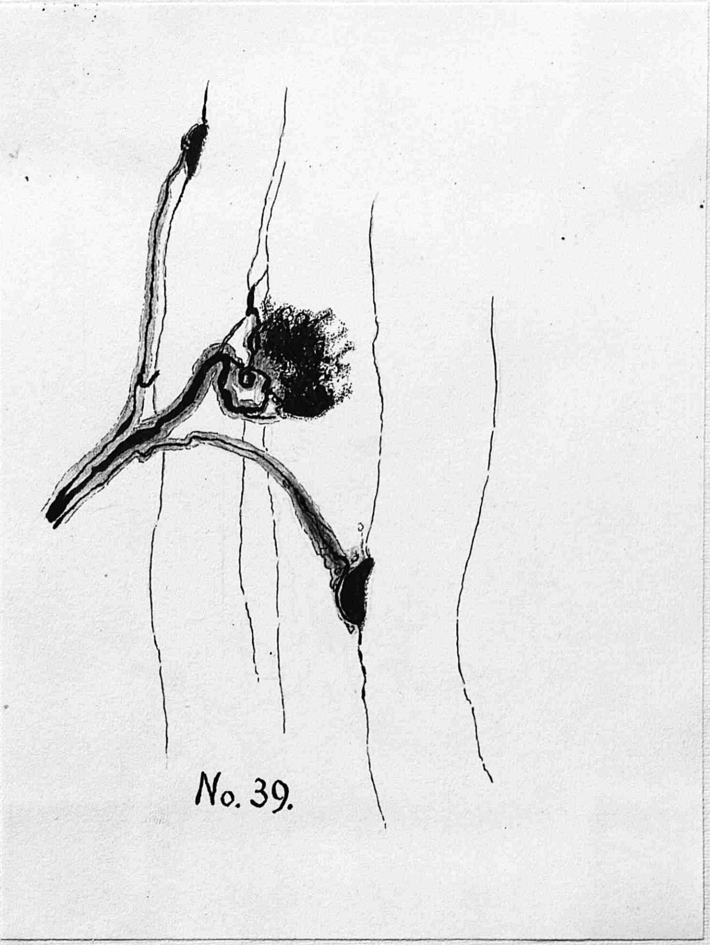


FIG. 3.

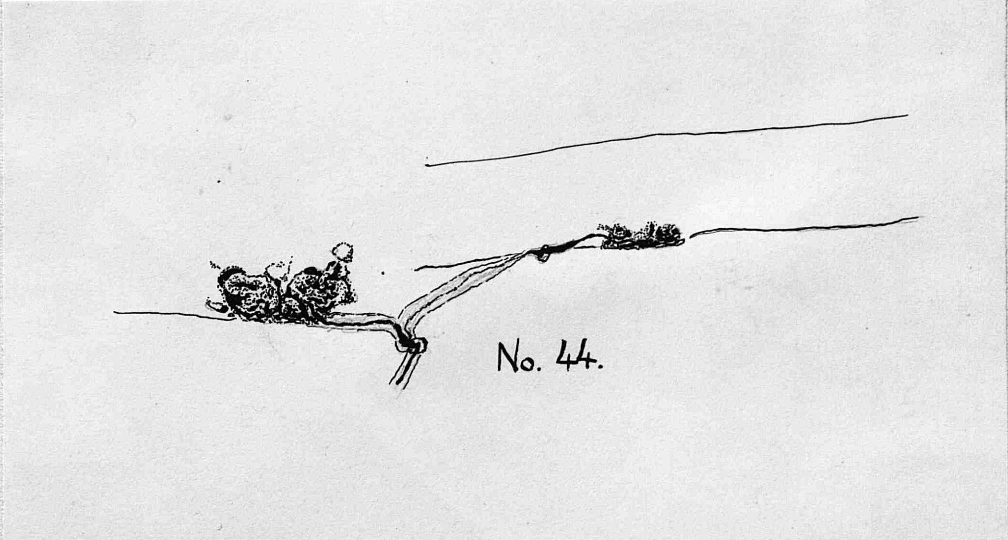


FIG. 4.

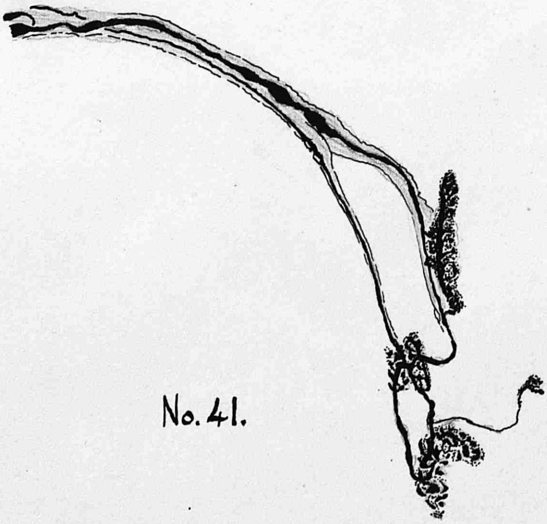


FIG. 5.

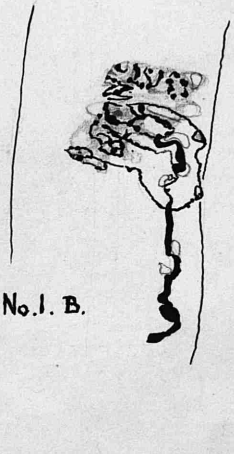


FIG. 6.

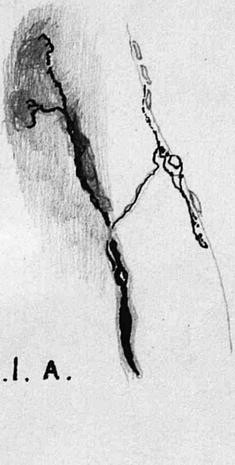


FIG. 7.

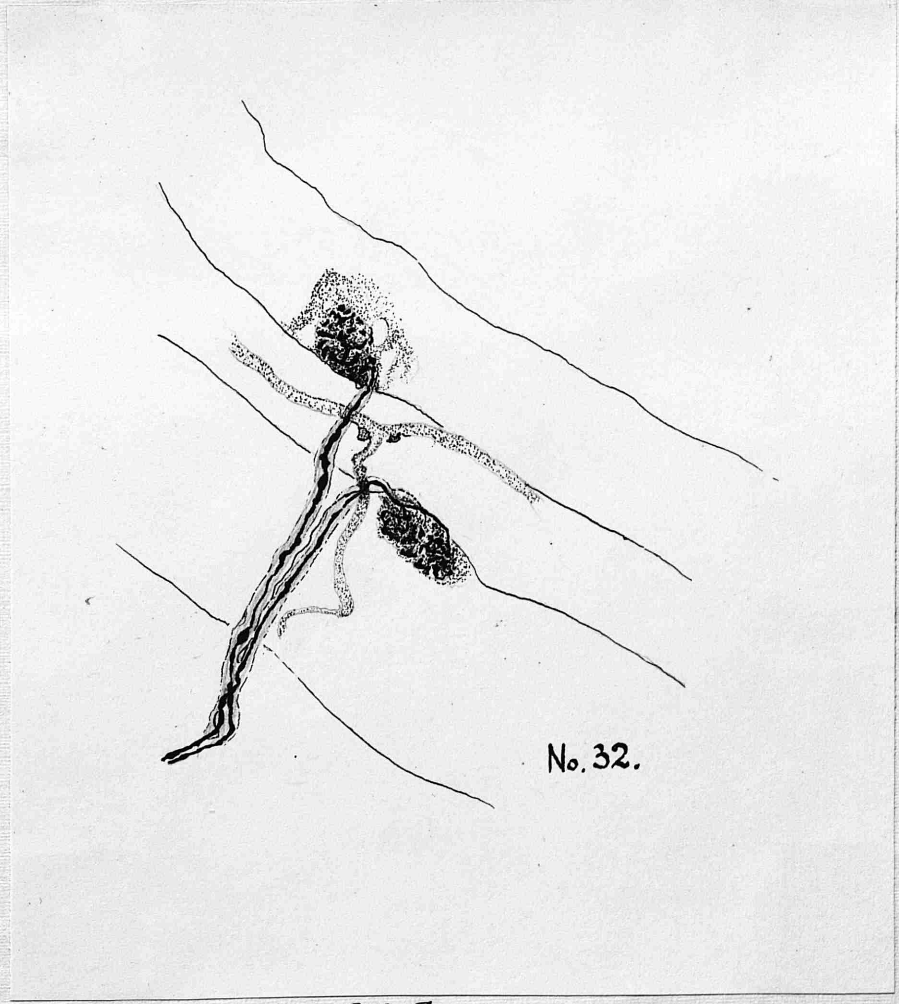


FIG. 8.

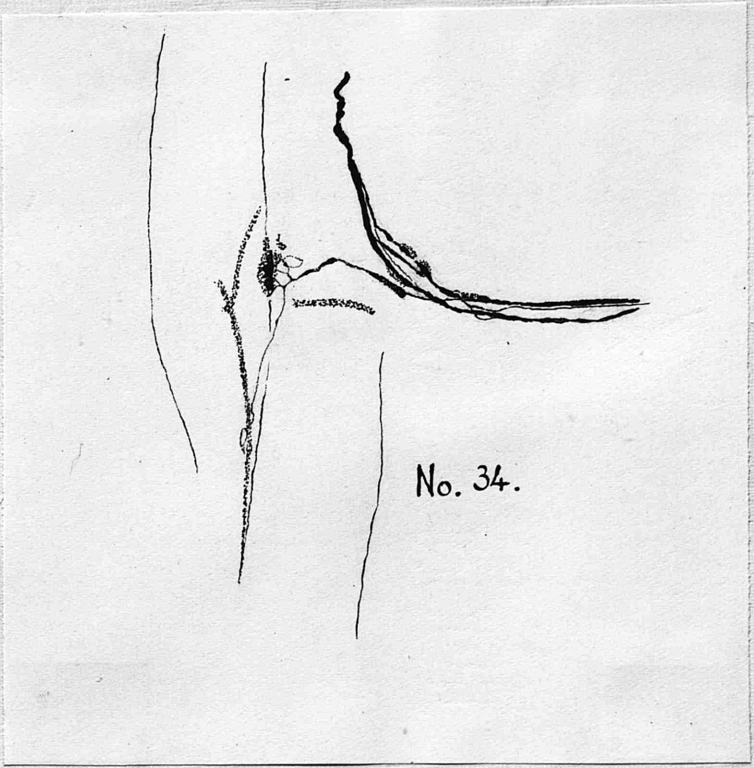


FIG. 9.

V

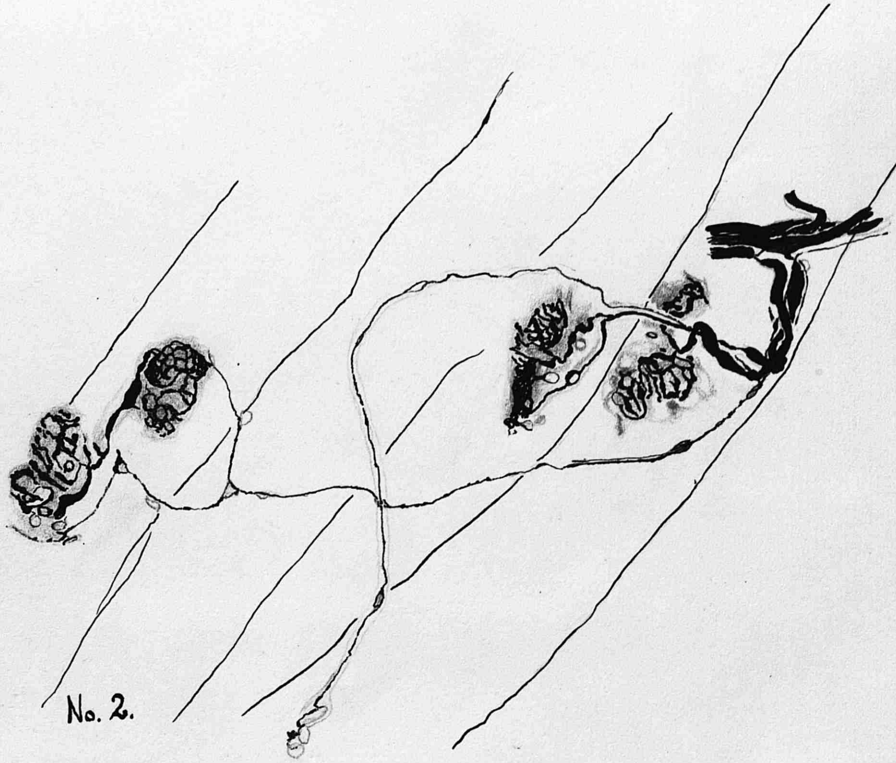
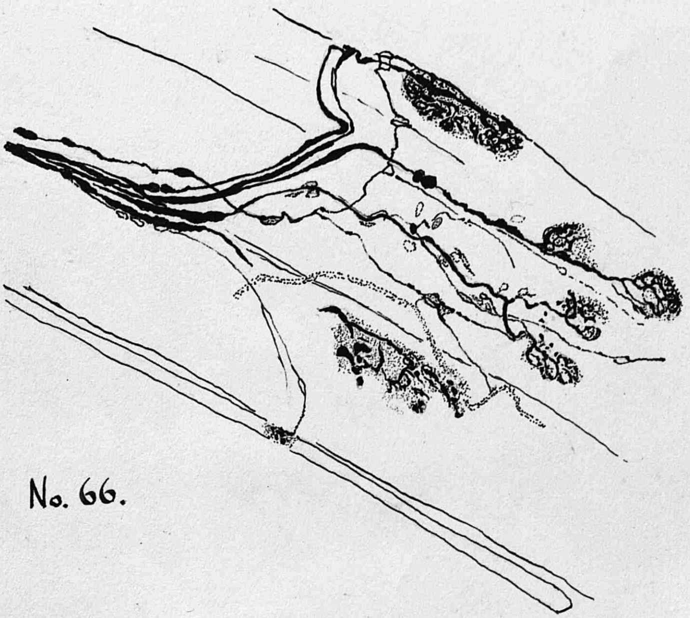


FIG. 10.



No. 66.

FIG. 11.

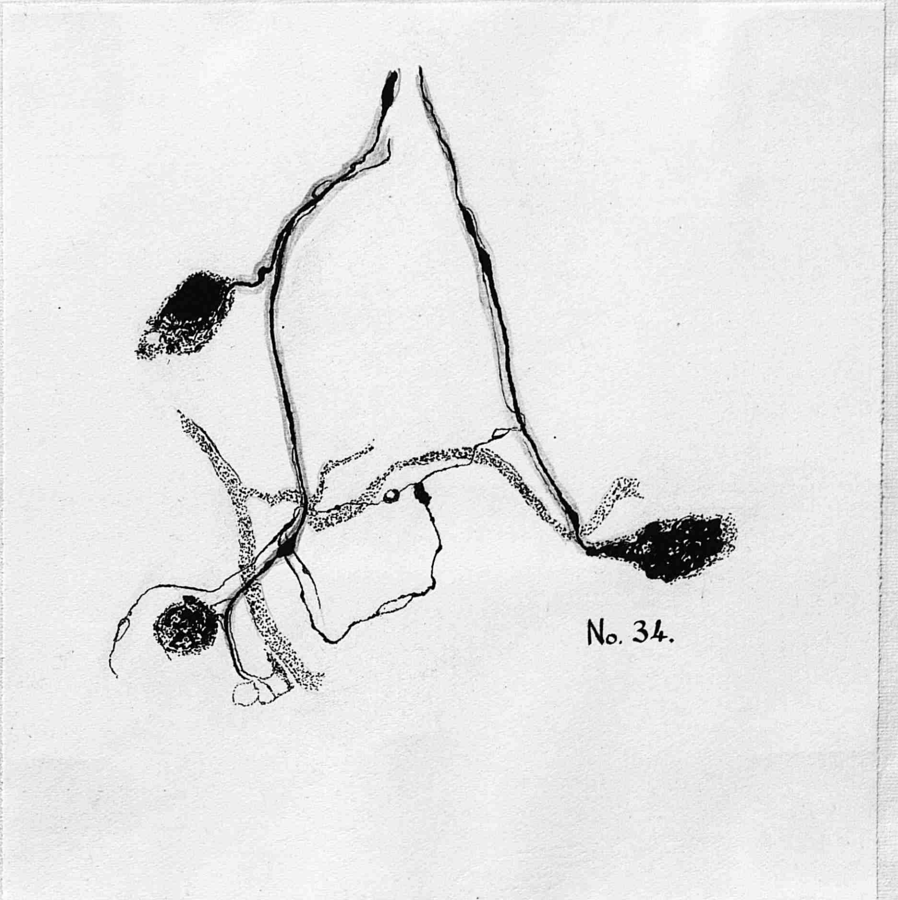


FIG. 12.

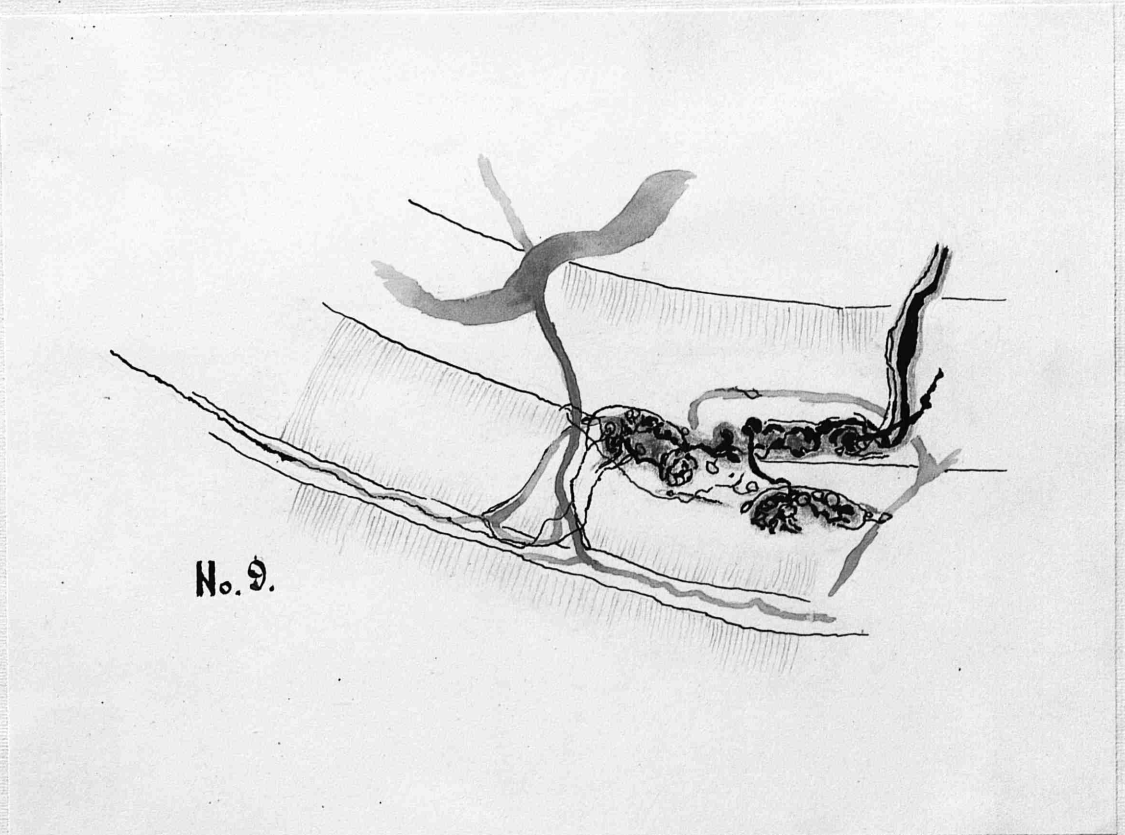


FIG. 13.

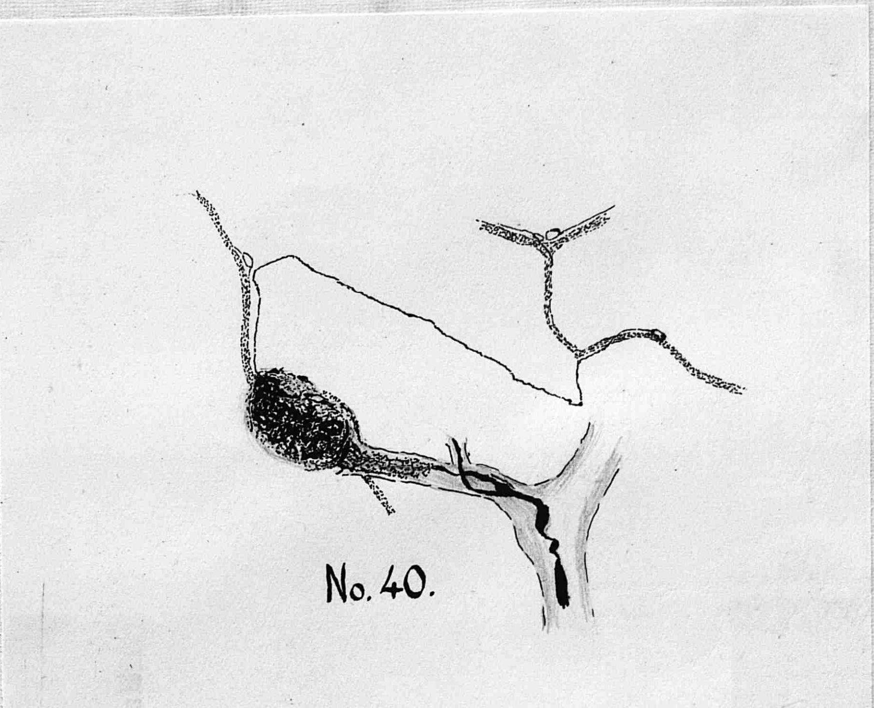


FIG. 14.

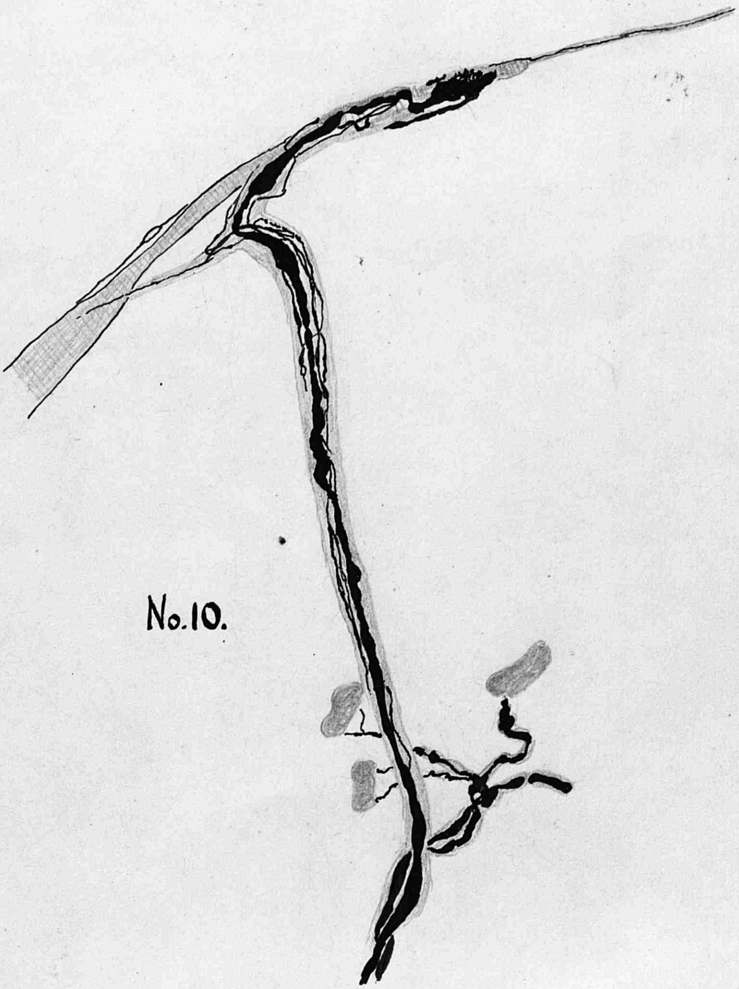
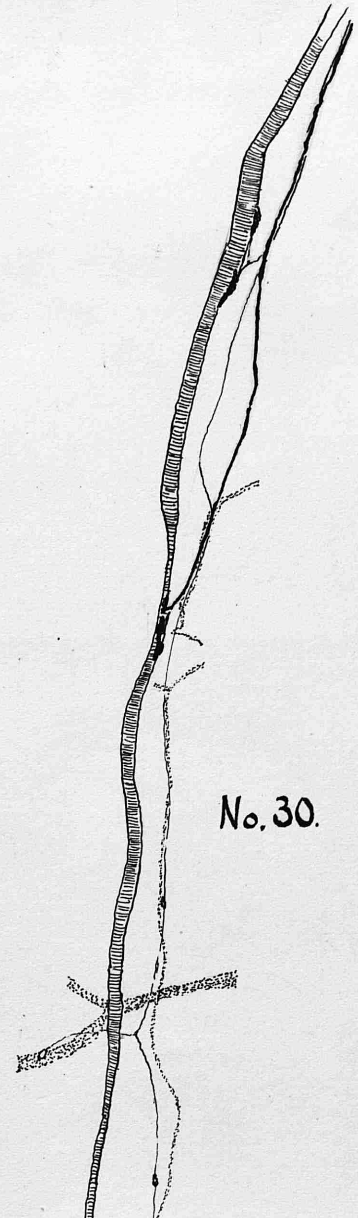


FIG. 15.

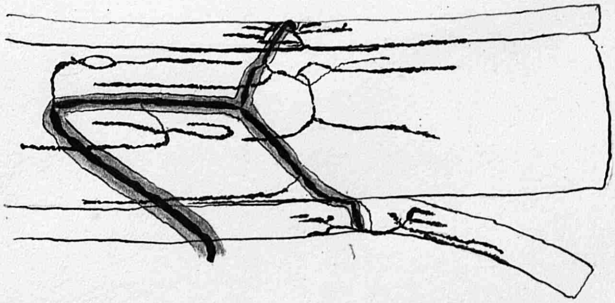
X



No. 30.

FIG. 16.

XI



No. 1.

FIG. 17.