Histology. — *"The innervation of the muscle-fibres of the myo-cardium and of the atrioventricular bundle of* HIS *in the heart of the tortoise (emys and cyclemys).* (1st Communication). By Prof. J. BOEKE.

(Communicated at the meeting of November 29, 1924).

In no organ the innervationproblem is of such a fundamental importance both for the physiology and for the pathology of it, as it is in the heart-muscle. In no organ, however, the histological study of the innervation meets with so many difficulties and obstacles as in the heart-muscle.

Although there is still some discussion going on about histological details, the mode of innervation of the cross-striated muscle-fibres, the form and distribution of the motor endplates on them, their connection with the muscle-fibres, are well known. In the heart-muscle it is not even known, whether there are distinct nerve-endings on the muscle-cells, or whether there is simply a plexus of nerve-fibres running between the muscle-fibres without distinct endings being formed. In his splendid and exhaustive treatise on the innervation of the heart [1]) published in 1908 MOLLARD draws the conclusion "qu'à l'heure actuelle on ne connaît pas encore d'une façon certaine et définitive le mode de terminaison des nerfs dans le myocarde. L'existence de terminaisons motrices établies sur le type de celles les plus simples des muscles striés ordinaires (RENAUT) n'est pas démontrée, non plus que l'existence d'un type spécial de terminaisons différent à la fois de celles des muscles striés qui sont enveloppées dans un sarcolemme, et de celles des muscles lisses (SMIRNOW)" (l. c. p. 185). And even to-day we have to confess that this statement is true.

It has been amply demonstrated for the different classes of the higher vertebrates, how extraordinarily rich in nerve fibres throughout its whole extent the myocardium is, but we are still quite ignorant about the way on which these nerve-fibres are connected with the contractile elements themselves. The majority of the investigators of the subject, and among them especially the authors, who have most thoroughly studied the innervation of the heart-muscle, lay much stress on the fact, that though it is possible to demonstrate a network of very fine nerve fibres surrounding the muscular elements on all sides, no trace of distinct free nerve-endings is to be found. The apparently free endings of the nerve fibres, which show themselves here and there in the preparations, must not be considered as real free nerve-endings, but as the results of a break in the impregnation of the nerve-fibres, a common feature of the

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[1]) J. MOLLARD. Les nerfs du coeur. Revue générale d'Histologie. Fascicule 9. 306 pages, 79 figures. 1908.

GOLGI-impregnation-method, consequently as a fault of the technique employed in the investigation, and not as a histological reality (HOFMANN). Even a pastmaster of the technique of the staining of the elements of the nervous system as DOGIEL only succeeded in demonstrating in the heartmuscle of the tortoise a very fine network of delicate nerve fibres surrounding the muscle cells, and finished his description of the mode of innervation by saying that in one of his preparations in one and only one place he had got the impression, that there were small knob-like nerve-endings to be seen [1]), attached to the surface of the muscle-fibres. The drawing which has to illustrate this point is however not convincing. MICHAILOW [2]) who in the year 1909 applied the impregnation-method of CAJAL to the heart muscle of the frog, described (just as JACQUES in 1902) distinct ramified nerve-endings on the muscle-cells of the heart, which he took for the endings of the vagus nerve; in addition to these endings he found again the same delicate varicose nerve-plexus surrounding the heart muscle-cells as described by former investigators. According to him however the existence of distinct end-branches, ending in a neurofibrillar endring or endloop on the surface of the muscular elements is undeniable. These endrings never enter the muscle-cells themselves. The earlier statements by RANVIER "que les fibrilles nerveuses pénètrent réellement dans les cellules musculaires et qu'elles passent au voisinage de leur noyaux" (1878, 1880) were the results of a defective technique and in consequence an incorrect observation.

Recent investigations have not been able to alter these views. The standpoint of the majority of the investigators to-day is essentially the same as was that of MICHAILOW and DOGIEL in the year 1907 and of GORDON WILSON in the year 1909. Extensive researches, it is true, on the minor details of the innervation of the cardiac muscle-elements, these later years did not bring us. The authors confirmed themselves to the investigation of the distribution of the ganglion cells in the wall of the heart, their connections with the cardiac nerves, their number in the different parts of the heart in different animals, and to the distribution and form of the sensory nerve-endings in the endocard and in the pericard.

This is not to be wondered at, for I don't know of any other tissue, which refuses so obstinately to take a nerve-stain as does the cardiac muscle. For years I have tried again and again to stain the nerve-fibres of the heart-muscle and their endings with the same method, which gave excellent results in the skeletal muscles of the self-same animals, but without success. It was only this year that by means of the neurofibrillar staining-method of BIELSCHOWSKY with a somewhat modified length of

[1]) J. DOGIEL. Einige Daten der Anatomie des Frosch- und Schildkrötenherzens. Arch. f. mikrosk. Anatomie. 70. Bd. 1907.

[2]) S. MICHAILOW. Internat. Monatschrift f. Anat. u. Physiologie. 25. Bd. 1909.

impregnation-time in the Ag NO_3-solution (6 to $6^1/_2$ days at a temperature of 35° C) I succeeded in getting good preparations with an excellent and elective colouring of the nerve-fibres and their endings in the cardiac muscle of the heart of tortoises and birds, and with tolerable results in the heart of the hedgehog.

In this communication however I will confine myself to the description of the distribution of the nerve-fibres and of the relations between the nerve-fibres and their terminal ramifications and the muscular elements, and the innervation of the muscular elements of the atrioventricular connection (bundle of HIS), in the heart of the tortoises (emys europaea, the European Pond-tortoise, and cyclemys sp.), because with those hearts I obtained the best and most complete results.

The form and arrangement of the elements of the cardiac muscle in the chelonian heart, their curious elongated and branched appearance, with the rod-shaped or oval nucleus in the middle, lying in a large quantity of granular sarcoplasm and surrounded by a ring of myofibrillae, which are freely continuous and pass over without interruption from one cell into the adjacent cells, is essentially the same in the heart of the lizard and in that of the cheloniae, and has been described by a number of authors. The arrangement of the muscular fibres, running in bundles, which are closely interwoven in the ventricular portion of the heart, but form a spacious network with thin strands of tissue and wide meshes in the auricular portion, may be considered also as sufficiently known after the descriptions given by BOTAZZI, DOGIEL, KÜLBES and LANGE, LAURENS and ENELLI. This arrangement too has essentially the same features as in the lizard heart, as it was described recently by KRAUSE (1923), and so there is no need to describe these things again here in full.

In preparations stained after the pyridine-silver method of BIELSCHOWSKY, treated afterwards with chloride of gold, and stained lightly with haematoxylin and eosin, the cardiac muscle-fibres show a very delicate but distinct cross-striation of the myofibrillae, and appear to be surrounded by a thin sarcolemma which is connected with the myofibrillae by very delicate but distinct cross-membranes of KRAUSE. The muscle cells are united by their branches into a syncytium without a trace of cellboundaries or junctional lines.

In the wall of the ventricle, as was stated before, the muscle-fibres are arranged in thick bundles, closely interwoven, and running in the sections apparently in all directions without any attempt at regularity. A closer study of serial sections however reveals a distinct regularity of arrangement. I may refer here to the excellent description given by BOTAZZI [1]).

The wall of the ventricle is not a compact structure. Everywhere it

[1]) F. BOTAZZI. Ricerche sulla muscolatura cardiale dell' Emys europaea. Zeitschr. . Allgem. Physiologie VI. 1907. p. 140—194.

consists of a network of closely interwoven bundles of muscle fibres, covered by the endocardium. These bundles however do not form generally a regular cavernous or sponge-like tissue, but they enclose a more or less spacious central cavity, which by means of the ostium atrioventriculare is connected with the cavity of both auricles, and surrounding it a system of additional cavities, so that the blood flows freely between all the bundles of muscle fibres and these bundles even quite near the outer epicardium-covered surface of the ventricular wall are lined by the endocardium.

In the auricles the system of trabeculae is much more spacious, so that the entire auricular wall is much thinner and the bundles are composed of a smaller number of muscle fibres and are lying more widely apart. Here too all the trabeculae are everywhere lined by endocardium, and in the sections the thinner bundles of muscle fibres are often seen to be connected here by thin bands, consisting only of endocardium without a trace of muscle fibres enclosed by it.

In the septium atriorum, the sinus venosus and the wall of the central ventricular cavity we find underneath the endothelial lining of the endocardium in thick bundles the curious layer of smooth muscle cells, described by GASKELL, ROSENZWEIG, BOTAZZI, which abuts on the tunica media of the large veins of the heart (c.f. LAURENS 1913) and which ROSENZWEIG, BOTAZZI and OINUMA held responsible for the oscillations of the tonus of the tortoise heart. LAURENS could find them only in the wall of the auricles, but without doubt they occur also in the wall of the ventricle. BOTAZZI compares them with the fibres of PURKINJE of the mammalian and avian heart.

Now in the first place we find even in the mammalian heart in the connective tissue underlying the endocardium-endothelium in places scattered bundles of non-striped muscle, as first pointed out by SCHWEIGGER-SEIDEL, which have nothing to do with the fibres of PURKINJE and the bundle of HIS-TAWARA. And secondly we find in the septum atriorum and the dorsal wall of the atria bundles of curious branched *cross-striated* muscle-cells (fig. 6), lying as a loose network in the connective tissue, which may be traced in the sections as an uninterrupted bundle into the wall of the ventricle, which with a better right may be compared with the bundle of HIS and the fibres of PURKINJE. But about this point we have more to say later on.

The innervation and the intracardial nervous system of the tortoise (and in general reptilian) heart has been described by a number of authors. For the description of the distribution of the nerves and their course in the heart wall as far as it is visible to the naked eye I can refer the reader to the paper by DOGIEL and ARCHANGELSKY [1]) and to

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[1]) Pflüger's Arch. f. d. Ges. Physiologie 113. Bd. U. 1.
J. DOGIEL. Arch. f. mikrosk. Anatomie 70. Bd.

the paper by LAURENS [1]). Nerves can be seen running along the superior venae cavae to the heart. On the right side a branch, the coronary nerve, runs directly from the sinus under the vein to the ventricle, the remainder of this nerve being distributed to the sinus and perhaps a small branch to the right auricle (LAURENS). The nerve on the right side is distributed principally to the left auricle from which can be made out several branches which run to the ventricle along the auriculo-ventricular groove, some ending here and others continuing on to the dorsal surface of the ventricle. Running to the ventral side of the heart are several fine branches which divide and are distributed to the different vessels of the bulbus aortae. The dorsal ligamentum atrioventriculare of DOGIEL contains one or more large nerve branches running to the ventricle, and numerous ganglion cells. According to LAURENS, whose description I follow here, groups of ganglion cells are more numerous in the tortoise heart than in that of the lizard. The largest are two at the opening of the sinus and of the pulmonary veins into the auricles. As was mentioned already, in the dorsal ligament there are numerous groups of ganglia all along the course of the coronary nerve. On the dorsal side of the left auricle, just under the pericardium, there are numerous small groups of ganglia to be seen, some consisting of only two or three cells. The same holds true for the wall of the ventricle. Here too there are a number of small subpericardial groups of ganglia, near the apex as well as near the coronary groove. At the beginning of the septum atriorum, on the right side, there is a collection of ganglia and also several small groups of nerve cells along this side of the septum. In the connective tissue of the auriculo-ventricular groove, particularly on the left side and in the proximity of the bulbus, the ganglionic masses are very numerous, though small, consisting of from two to five cells (LAURENS). In BIELSCHOWSKY-preparations the plexus around the ganglion cells appears to be very rich. By that reason it is impossible to trace distinct nerve-fibres to separate ganglion cells, or from them to the separate muscle fibres. Experimental work (cutting of the vagus and sympathetic nerve, etc.) may perhaps throw some light on this question too.

For the question of the finer details of the innervation the distribution and the course of the nerve-fibres as seen in the sections under the microscope is of more importance.

As it was described already years ago by GERLACH, the nerve-fibres of the heartwall form everywhere a network, a plexus. Large nerves do not enter into the heart-muscle, except the larger nerve-branches, described above, which originate from the groups of ganglia in the septum atriorum and the neighbourhood of the large veins of the heart and pass from here to the wall of the auricles and of the ventricle.

The ramifications of these nerves form a dense nerve-plexus under the endocardium (the "groundplexus" of GERLACH and HOFFMANN).

[1]) Anat. Record. Vol. VII.

This groundplexus is very rich, and consists of coarser nerve-strands in the wall of the ventricle, and of very fine nerve-threads in the trabeculae of the auricles. From this fundamental sub-endocardial plexus arise numerous fine fibres, which terminate at different levels of the endocardium in more or less complicated sensory nerve-endings. The heart is, just as in the higher vertebrates, supplied as abundantly with afferent nerves as is the most sensitive skinsurface.

But in this groundplexus are running also the efferent nerve-fibres, and indeed, when we study the plexus there where it lies under the endocardium of the trabeculae carneae of the auricles, or of the finer muscle-bundles of the ventricular wall, we get the impression that this nerve-plexus, whose branches, consisting of very fine nerve-threads, are seen running in different directions even on the smallest trabecles of the atria, is chiefly of an efferent nature, as will be discussed below.

There is no part of the muscle-bundles to be found, where this subendocardial nerve-plexus is absent. We find it even on the smallest muscle-columns of the auricles and of the ventricle, and even there, where as was described before, two of the trabeculae carneae of the auricles are connected by a strand, consisting only of endocardial tissue, without a single muscle-fibre in it, we often see a very delicate neurofibrillar strand running inside this endocardial thread and connecting the groundplexus of one trabecle with that of the other.

Whether the branches of this nerve-plexus form closed meshes or not, is difficult to state. Everywhere we see the neurofibrillar strands branching and ramifying on the surface of the muscle-columns, but where in the sections in most cases only fragments of the plexus are to be seen, it is difficult to state, whether branches of the neurofibrillar strands, once parted, come together again to unite into one single branch, or simply run across each other in different directions.

In a general way the drawing of the groundplexus around the trabecles of the auricle of the frogs heart, given by HOFMANN [1]), furnishes also a good picture of the features of this plexus in the heart of the tortoise.

From this fundamental subendocardial plexus we see everywhere fine neurofibrillar strands passing into the muscle-columns. They are running here at first between the muscle-cells, as it is to be concluded from the study of cross-sections of the muscle-columns and from the fact, that in longitudinal sections of a muscle-bundle these nerve-fibres are often seen running at right angles to the long axis of the muscle-fibres, winding their way across two or three muscle-fibres. But when we follow these neurofibrillar strands during their course, we see them curve round very soon

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[1]) F. B. HOFMANN. Das intracardiale Nervensystem des Frosches. Arch. f. Anat. und Physiol. Anat. Abtheilung 1902. Tafel IV.

F. B. HOFMANN. Histologische Untersuchungen über die Innervation der glatten und ihr verwandten Muskulatur der Wirbeltiere und Mollusken. Arch. f. mikrosk. Anatomie, 70. Bd. 1907.

and run on in the direction of the muscle-fibres. These nerve-fibres which are running parallel to the long axis of the muscular elements are nearly without exception varicose, the varicosities often following each other so regularly in the course of the nerve-fibre as to suggest the comparison with a string of beads.

Everywhere we see these delicate varicose threads running through the muscle-bundles, and in the straight long muscle-bundles we often meet in the sections through the wall of the ventricle, they are often to be followed a long way passing a number of muscle-nuclei. In the groups of muscle-fibres fixed in a contracted state they often appear as meander-like winded threads. Especially in the sections through the wall of the auricles the short thin muscle-columns running freely through the atrial cavity and often being composed of only two or three muscle-fibres, present even in thin sections (15—25 μ) a very clear picture of the groundplexus underneath the endothelial lining and of the nerve-ramifications between the muscle-fibres inside.

Whether these different intermuscular branches are still running exclusively between the muscle-fibres or are lying already imbedded in the protoplasm of the fibres themselves is not easily to determine in each case given. But it is certain, that *from these intermuscular nerve-threads branch off very fine varicose nerve-fibres, which pass into the muscle-cells and lie imbedded in the protoplasm itself.* They are extremely delicate, beset with small *irregular* varicosities, and either may be followed through two or even three muscle-cell territories, or end abruptly within the sarcoplasm with a small endnet or endloop or a series of terminal varicosities appearing as very small ring-like expansions of the neurofibrillar structure. In fig. 1 several of these nerve-endings are drawn from a section through the auricles, in fig. 2 a cross-section through part of a muscle-column of the ventricle shows the outline of some of the muscle-fibres, the one in the middle showing the nucleus and lying close to it imbedded in the sarcoplasm a small neurofibrillar ring at the end of an extremely delicate neurofibrille coming from the outer part of the muscle-fibre.

The clearness and distinctness of the impraegnation of the neurofibrillae enabled us to ascertain with the utmost surety, that we had before us real endings of the neurofibrillar structure and not varicosities cut off by the microtome-knife. And indeed, we find those endings everywhere in the preparations, and where they are lying in the middle part of the sections, so that they are covered above and below by a layer of tissue, we may quite easily ascertain, whether they are real endings with rounded endloops or simply a place, where the neurofibrillar strand was cut across. Especially in the thin muscle-bundles of the auricles the intraprotoplasmic neurofibrillae with their varicosities and small endnets or endrings were distinctly visible as such. In many cases the intraprotoplasmatic position of these neurofibrillar endings is made clear by their lying in exactly the same niveau as the nucleus in the clear nearly

uncoloured protoplasm, which surrounds the nucleus especially at both poles and which is surrounded by a ring of cross-striated myofibrillae. Inside this ring we often find the endloops of the neurofibrillar strands. This appears evident in those cases, as are drawn as exactly as possible in figg. 2, 4 and 5, where the neurofibrillar endring is lying quite close to the nucleus, sometimes even enclosing the top of the elongated nucleus. In this regard fig. 5 seems important; here a reticulated varicosity of the neurofibrillar strand was lying so close to the nucleus of a muscle-fibre, that it is making an indentation in the nucleus, and thus was lying in a cavity so deep, that at first sight it made the impression as if two nuclei were present, the neurofibrille passing between them. Only by focussing very carefully it became clear, that there was only one nucleus present, and that the neurofibrillar ring was lying close against it. Another endring too is shown in the same figure, lying also close against the side of the same nucleus. In cross-sections through the muscle-columns we see that the nerve-fibres, which run in the direction of the long axis of the muscle-fibres, are lying as a rule in the peripheral layer of the sarcoplasm, and one is tempted to ask, whether the principal intramuscular fibres, which were described as running with many curves apparently between the muscle-fibres, are in reality all of them lying between the muscle-fibres, and whether they are not, at least a number of them, already enclosed in the protoplasm of the muscle-fibres themselves.

The delicate varicose neurofibrillar threads furnished with endrings and endloops, whose intraprotoplasmic position could be made out convincingly, showed here and there a distinct connection with the sarcoplasm, an extremely delicate network, at one side continuous with the neurofibrillar structure, at the other side apparently passing into the cross-striation of the myofibrillae, at least with meshes, which in size correspond with the width of the cross-striation. In conformity with what is known about the motor-endplates on the cross-striated muscle-fibres of the voluntary muscles and their protoplasmic connection with the myofibrillae, it would be possible to identify this network with the *periterminal network* of the striped muscle-fibres.

In short, we find everywhere in the cardiac muscle an innervation of the individual contractile elements, consisting not only in a pericellular network, which encloses the muscle-fibre without definite nerve-endings (HOFMANN), but in a form, which on one hand reminds us of the mode of innervation of the non-striped muscle-fibres, as it was described for example in the musculus ciliaris of the human eye [1]), on the other hand exhibits some features which remind us of the structure of the motor endplates on the striped muscle-fibre.

And finally, our observations seem to confirm to a certain degree the old statement by RANVIER, which was cited in the beginning of this

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[1]) J. BOEKE. These Proceedings 17, p. 1982 and 18, p. 2.

paper, "que les fibrilles nerveuses pénètrent réellement dans les cellules musculaires et qu'elles passent au voisinage de leurs noyaux".

Auriculo-ventricular bundle (bundle of HIS). According to DOGIEL and ARCHANGELSKY (for the tortoise) and IMSCHANITZKY (for the lizard) the different parts of the reptile heart are not connected muscularly, the connection between the auricles and ventricle being effected solely by means of a large nerve bundle which runs in a band of connective tissue on the dorsal side of the heart, the "ligamentum atrioventriculare" of DOGIEL (1906).

It seems however, that the contradictory statement by a number of authors (f. ex. BOTAZZI, ROSENZWEIG, KÜLBS and LANGE, LAURENS) is convincing, and the existence of a funnel-shaped muscular connection between the auricular wall and the ventricle seems to be demonstrated beyond doubt.

But, as I mentioned before, it seems to me that two phenomena, which have nothing to do with each other, are often mixed up together in this respect. By BOTAZZI, in his paper cited on a previous page, much importance for the co-ordination of the heart is ascribed to the subendocardial layer of smooth muscle-cells described by him and by ROSENZWEIG and which forms a continuation of the tunica media of the large veins of the heart into the auricles and the ventricle [1]), and when I understood rightly the short description, insufficiently illustrated, of KÜLBS and LANGE, these authors have the same elements in view, when they describe the voluminous funnelshaped muscular connection between the auricles and the ventricle. Now, as is pointed out by LAURENS, there exists without doubt a smaller funnelshaped connection between the auricles and the ventricle, consisting of *cross-striated muscle-fibres*, with a distinct but fine striation and large round or slightly oval nuclei, and of a brighter appearance than the ventricular fibres. This is the real bundle of HIS. It is thickest at the dorsal side of the auricles in the hind part of the septum atriorum, where we find the ligamentum atrioventriculare of DOGIEL, which forms a pathway for nerves and blood vessels and groups of ganglion cells between the sinus and the ventricle. This muscular connection, the real bundle of HIS, is composed of cross-striated muscle cells (c. f. LAURENS), which are more loosely arranged in the connective tissue than the muscular elements of both auricles and ventricle, and therefore present a more branched and netlike appearance (fig. 6), herein exhibiting the same features, which are so characteristic for the fibres of PURKINJE in the avian and mammalian heart. At one side this muscular bundle is connected with the auricular musculature, at the other side with the inner wall of the ventricle.

The striated muscular elements of this bundle receive an individual

[1]) According to LAURENS they occur only in the auricles, but they are distinctly visible also in the wall of the ventricle enclosing the central ventricular cavity.

J. BOEKE: "The innervation of the muscle-fibres of the myocardium and of the atrioventricular bundle of His in the heart of the tortoise (emys and cyclemys").

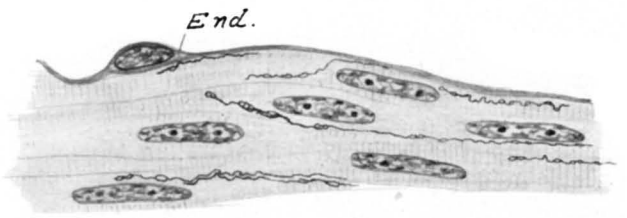

Fig. 1. Nerve-endings in a longitudinal section through a muscle-trabecle of the auricle of the heart of emys europaea.
End. = Endocardium.

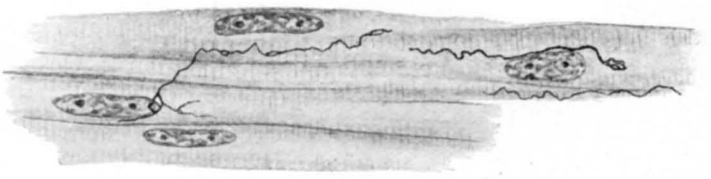

Fig. 2. The same from the wall of the ventricle.

Fig. 3. From a cross section through a muscle-column of the auricle, showing the nucleus of one of the fibres, and besides it an intraprotoplasmic neurofibrillar endring (foreshortened).

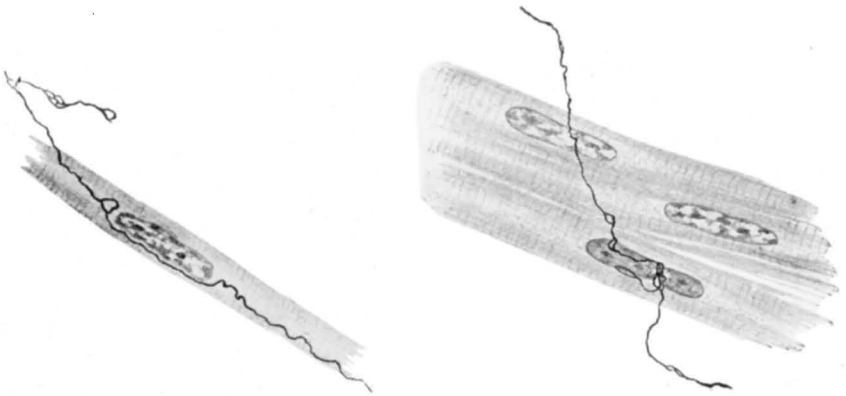

Fig. 4 and 5. Heartmuscle-fibres from the wall of the auricle of the tortoise heart with neurofibrillar nets lying close to the nucleus.

Fig. 6. Longitudinal section through the connective tissue of the septum coronarium with loosely connected muscle-fibres of the atrioventricular bundle of HIS, and on these fibres small terminal nerve-fibres *(nf.)*.

efferent innervation in the same way as it was described in the foregoing pages for the muscle-cells of the auricles and the ventricle. Not only we find, running along it, the large nerve-bundle described above, and in connection with this numerous groups of ganglion cells, but the muscle-elements themselves possess an individual innervation (fig. 6), intraprotoplasmic nerve-fibres with neuro-fibrillar endrings and varicosities (fig. 6 *nf*), and a perimuscular nerve-plexus, which is certainly not less developed than around and in the muscle-columns of the auricles and the ventricle. Every individual muscle-fibre of the bundle of HIS is innervated.

In conclusion we may remark, that also in the subendocardial layer of smooth muscle-cells of BOTAZZI and ROSENZWEIG referred to above, a plexus of very delicate nerve-fibres with varicosities and small endnets could be traced throughout the whole layer. The strands of this plexus appeared to be in connection with the perimuscular nerve-plexus of the muscle-bundles lying underneath and seemed in many cases to be the direct continuation of this plexus.

Utrecht, November 1924.