Resumen por el autor, Lee D. Cady.

Un estudio microscópico del fascículo senoventricular del corazón del conejo, con referencia a los datos relativos a su interpretación funcional, especialmente en términos de un manantial de reemplazo del miocardio degenerado.

El nodo del seno y el nodo atrioventricular son prácticamente idénticos en estructura histológica en el conejo. En estos nodos puede observarse la presencia de numerosos núcleos en varios estados de división amitótica, pero los núcleos de las células binucleadas están colocados muy próximos entre sí y no existe prueba de la división protoplásmica. La porción principal del fascículo atrioventricular presenta una estructura muy semejante. En las fibras de Purkinje, sin embargo, los núcleos están más separados que en las porciones del sistema conductor situado más encima. En las áreas de transición entre las fibras de Purkinje y el miocardio ventricular, se encuentran tan solo núcleos impares. En el tejido nodal o en el tejido del fascículo no existe indicio alguno que indique la división citoplásmica de las células, ni tampoco hay dato histológico que indique un reemplazamiento de las fibras miocárdicas degeneradas por una progresión genética de las células a partir del nodo del seno hacia el miocardio ventricular.

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A MICROSCOPICAL STUDY OF THE SINOVENTRICULAR BUNDLE OF THE RABBIT'S HEART; WITH REFERENCE TO THE DATA RELATIVE TO ITS FUNCTIONAL INTERPRETATION, ESPECIALLY IN TERMS OF A SOURCE OF REPLACEMENT OF DEGENERATED MYOCARDIUM

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ONE PLATE (FIVE FIGURES)

CONTENTS

Introduction.....	375
Materials and methods.....	376
Description.....	378
Discussion.....	380
Summary.....	385
Bibliography.....	385

INTRODUCTION

This study of the sinoventricular bundle was undertaken with the view of testing Retzer's ('20) recent rather startling conclusion that worn out myocardium is replaced from the sinus region of the heart, the new myocardial fibers being derived from the Purkinje fibers, which in turn are said to be derived from higher regions of the sinoventricular system. He compares the sinus region to the basal layer of cells in stratified (transitional) epithelium in that both of them are differentiated in the embryo, and he thinks that the sinus cells continue to multiply in a manner analogous to the manner in which the basal cells of stratified epithelium multiply in adult life. In 1908 Retzer expressed his belief that the Purkinje fibers are derived from the sinus tissue, but said nothing about a possible derivation of myocardial fibers from the Purkinje fibers. Retzer's conclusion is based for the most part upon a study of the pig's heart.

That there is a direct transition between the Purkinje fibers and the myocardial fibers was shown by Tawara ('06), and confirmed by Retzer ('08), Lhamon ('12), DeWitt ('09), and others, who traced the Purkinje fibers to their transition into cardiac fibers; but none mentioned indications of a possible replacement of degenerated myocardial fibers from that source.

This paper is concerned with the study of the rabbit's heart only with reference to the sinoventricular bundle, as Retzer terms the entire conduction system from sinus to Purkinje fibers, inclusive, and purports to present the data as found in the material studied with reference to its functional interpretation, especially as regards the replenishment of worn-out myocardial fibers by the sinoventricular bundle. No attempt was made to study the 'right lateral connection' of the atrioventricular junction, as Kent ('13) calls it, for its functional existence has apparently not been substantiated (Erlanger, '08). Retzer ('08) describes it in the pig's heart. We have been unable to find any really clear clinical demonstration that such a connection exists, and the experiments of Erlanger and Blackman ('10) show conclusively that there is either no such connection or that, if there is such a connection, it does not function as a pathway of conduction when there is greatest physiological need for it, namely, when the sinoventricular system is destroyed.

MATERIALS AND METHODS

The heart of the rabbit shows the conductive system with extraordinary detail in routine preparations from some of the specimens, there being some variation in this respect. About twenty rabbits' hearts were studied. They varied in age from one week to the well-matured specimens that can be found in the ordinary animal room. Sections were also kindly furnished by Dr. H. E. Jordan, which were invaluable in carrying out this work. Altogether something over three thousand sections were examined.

Details regarding technique were taken from Jordan's 'Text Book of Histology' ('20). A variety of fixing methods were used, including Flemming's, Meves', Zimmermann's, Zenker's,

Zenker-formol, 95 per cent alcohol, and 10 per cent formalin. One adult heart was opened by a longitudinal incision through the lateral wall of the right atrium and the cut continued down through the ventricle until the apex was reached. The parts of the lateral walls of both atrium and ventricle were then pulled aside and the whole heart flattened with thin strips of cork against a clean glass slide, weighted down in that condition with light weights, and covered with Zenker-formol fixing fluid. By this method it was possible to include in the longitudinal sections of the heart longer sections of the conductive system. A one-week-old heart, fixed in 95 per cent alcohol, was sectioned transversely; i.e., at right angles to the long axis of the heart. The sections were mounted serially and stained with hematoxylin and eosin. The atrial septum of a young adult, likewise fixed in 95 per cent alcohol, was sectioned longitudinally and mounted serially, beginning with the endocardial surface of the left atrium, and stained with hematoxylin and eosin. These sections were designed to show the nodal tissue. Blocks of the septum and of the walls of both ventricles were sectioned longitudinally, in order to get longitudinal and oblique sections of the transition areas of the Purkinje fibers. The material furnished by Doctor Jordan, a celloidin preparation, fixed in Zenker's fluid, was cut transversely, and stained with hematoxylin and eosin.

All serial sections were mounted five or six to the slide, and the sections on alternate slides were stained with hematoxylin and eosin, while the remaining sections were stained either with iron hematoxylin and picro-fuchsin or by other special methods, including Mallory's phosphotungstic acid-hematoxylin. Blocks of tissue from two hearts were stained with hemalum after Zimmermann fixation for intercalated discs. Silver impregnation methods were also used. All sections were mounted in Canada balsam.

DESCRIPTION

Keith and Flack's ('06) description of the sinus node in the human heart agrees closely with what is found in the rabbit's heart. They describe the nodal fibers as striated, of fusiform shape, with well-marked elongated nuclei, plexiform in arrangement, and imbedded in densely packed connective tissue—in fact, of a structure very like that of the atrioventricular node. There is slightly more connective tissue in the atrioventricular node in our sections than in the sinus node (figs. 1 and 2), but this tissue is not abundant enough to warrant the description as 'closely packed.' The nuclei could hardly be described as 'well-marked elongated nuclei,' for they are more nearly oval. A careful comparative study of the two nodes in the sections fails to reveal any distinct difference other than those already mentioned. They further state that these two nodes resemble each other in other mammalian hearts, the resemblance being especially marked in the mole, rat, and the ram. To this enumeration we can add that they are practically identical in appearance in the rabbit.

In the atrioventricular node (fig. 2) and the sinus node (fig. 1) the cross-striations of the fibers appear only very faintly in some of the hearts. In others the striation is more conspicuous. The myofibrils are peripherally placed; the nuclei are located centrally and they are surrounded by a relatively large amount of undifferentiated sarcous material which is rather granular. Frequently, however, in the regions of the nuclei there is no evidence of granules in the stained sections. Only rarely can one find any fibrils immediately adjacent to the nuclei. The nuclei are large, oval, and rather vesicular in appearance. They contain scattered granules of chromatin material which, except for a few chromatin clumps, is pretty evenly distributed. Many of the nuclei show one or two nucleoli. Frequently one finds two nuclei, rather smaller and more heavily stained than the larger single nuclei within the same perinuclear spaces and in close juxtaposition. No evidence of mitotic division can be seen in the cells of either of these nodes. Connective-tissue cells are scattered rather sparsely throughout the sectioned surfaces of

either node, but slightly more connective tissue is in evidence in the atrioventricular node. There seems to be no definite connective-tissue sheath surrounding either node. The nodal fibers of the sinus pass by gradual transition into the atrial fibers and are soon lost in them. The two nodes resemble each other so closely that one is safe in saying that they are identical in appearance, for, unless one knows the exact region of the section, it is impossible to identify either node with any degree of certainty except by the relative amount of connective tissue, which is in itself a variable (Cohn, '09).

The cells of the sinoventricular bundle below the region of the sinus node, that is, in the atrioventricular node, have very well-defined boundaries (fig. 3). The cytoplasm in stained sections appears pale, and in many of the cells shows only slight differentiation. The cell peripheries stain more deeply than the remainder of the cytoplasm, and at the levels of the nuclei show peripherally placed myofibrils, which are finer than those of the myocardial fibers. The nuclei are usually situated in relatively large clear areas and in the central portion of the cells. In fact, the large clear central area is the most conspicuous feature of these cells. The nuclei are rather pale, but well stained. Chromatin granules are fine and pretty evenly distributed, but nuclei are easily found that show chromatin clumps as in the atrioventricular node. Well-defined nucleoli, which take a pink stain in the hematoxylin and eosin preparations, are found in the nuclei; indeed, many nuclei show from two to four nucleoli. A large percentage of the cells have two nucleoli.

One cell was noticed that appeared to have four nuclei within the same perinuclear space. Where there are two nuclei, they are usually somewhat smaller than the nuclei in the mononucleated cells and they are darker in appearance. The binucleated cells also have nuclei that closely resemble the single nuclei in size, shape, and staining reaction. This type of nucleus is generally less closely apposed to its fellow than are the smaller and darker nuclei, which are usually somewhat flattened against each other. Nuclei in the various stages of amitotic division are frequently seen. However, no cells were seen that had their

nuclei widely separated or showed evidence of cytoplasmic division. No mitotic figures were seen in any portion of the system. The bundle is separated from the ventricular myocardium by means of a thin connective-tissue sheath. Connective-tissue cells are found also scattered throughout the entire bundle. The bundle has a subendocardial position everywhere except in the moderator band and in the so-called 'false tendons.'

In the case of the moderator band and of the false tendons (fig 3) the above description of the bundle also applies except that these particular subdivisions of the sinoventricular bundle are still further subdivided by connective-tissue septa, delicate in the moderator band, relatively robust in the false tendons, where the larger strands of the bundle are enveloped by relatively dense connective-tissue sheaths.

The Purkinje fibers (figs. 4 and 5) are only slightly more differentiated than the bundle cells. The nuclei are of about the same size and appearance as those of the bundle except at the points of transition into the myocardial fibers where they are smaller and gradually pass over into the myocardial type of nuclei. As the transition areas are approached, the binucleated cells become progressively fewer until the transition area is reached, where only unpaired nuclei are found. The cytoplasmic transition is more gradual than that of the nuclei, and it is almost impossible to determine just where Purkinje fibers actually end and the myocardial fibers begin. The Purkinje fibers are larger than the myocardial fibers, but at the transition points they diminish in diameter gradually and take on a more conspicuously striated and fibrillated condition. The connective-tissue sheath of the Purkinje fibers is very delicate and often does not show, especially at the transition points.

DISCUSSION

To answer the question whether the sinoventricular bundle consists of embryonal tissue and whether it may be able to differentiate into myocardium, one must consider its histogenesis. The cells of the sinoventricular bundle are differentiated early in embryonic life (eleven weeks in Keith's human embryo). The

facts that their myofibrils are more delicate and more peripherally placed and less conspicuously striated than in the myocardium and that there is more undifferentiated sarcous material centrally in these cells do not argue conclusively that they are embryonic cells. Their large size, their variously stout fusiform, polyhedral, and irregular shapes, and their striated fibrillar structure, all jointly indicate that they are specialized cells. Lange ('14) arrives at the conclusion also that the Purkinje fibers are not embryonic remains, since they are clearly distinguishable in very young mammalian hearts. He concludes further that they are a non-nervous apparatus for conducting the impulse to heart beat. The most suggestive evidence in favor of Retzer's conclusion relates to his observation that in the cells of the terminal portions of the bundle the nuclei have moved relatively farther apart. But this fact may be equally plausibly interpreted as the result of a secondary (mechanical) elongation of the cells of this region consequent to the imposition of stresses during the growth of the heart.

Howell ('19) states that it is uncertain whether the atrio-ventricular bundle contracts during systole, but that there is little doubt as to its function of conduction of the impulse to heart beat. Intercalated discs are not found in the atrio-ventricular bundle, but they are present in the Purkinje fibers (Jordan, Banks, '17). They occur very early in fetal life in the myocardium, during the second month in the heart of the beef (Jordan, Banks, '17). Assuming that intercalated discs are modified irreversible contraction bands produced through functional strains and stresses, we come to the conclusion that the atrioventricular bundle either does not contract, or at least; if it does contract, it does not share the strains ordinarily incident to heart beat, and that the Purkinje fibers do contract. However, the cells of the bundle show cross-striation, which is in itself an indication of their muscular nature. Curran's ('09) conclusion is that the presence of the constant bursa (interpreted by Lhamon ('12) as a connective-tissue sheath and not as a bursa) which he describes surrounding the atrioventricular bundle indicates that this muscle bundle either does not contract at all or that it con-

tracts in a different way and at a time which is not synchronous with the contraction of the ventricular myocardium, and he inclines to the latter view. His observation on a beating heart lead him to believe that the atrioventricular bundle contracted synchronously with the atria, but his observation was not confirmed by graphic methods. Erlanger ('12) was not able to demonstrate any contraction in excised segments of false tendons even when they were perfused through their own blood vessels with various solutions. The fact that there are no intercalated discs in the atrioventricular bundle does not prove that it does not contract, nor does the fact that there are cross-striated myofibrils in the bundle prove that it does contract. Embryonic hearts, and tissue cultures from embryonic chick hearts, exhibit rhythmical contraction before intercalated discs or the full complement of cross-striations appear. Cross-striations are apparently not necessary for contractility in heart muscle. Doctor Burrows kindly made it possible for us to examine some of his tissue-culture preparations of embryonic chick hearts ('12). We found no intercalated discs nor cross-striations, but only irregularly arranged myofibrils within the sarcoplasm. These preparations were known to have shown rhythmical contraction for relatively long periods of time. It may be possible that the sinoventricular bundle conducts the impulse to heart beat and still does not itself contract. If the calcium be removed from one end of a strip of myocardium and that end be stimulated, it will conduct the impulse for contraction to the untreated end, which immediately contracts, but the treated end will not contract. A knowledge of the calcium content of the bundle would be interesting in this connection. However, the only conclusive method of determining whether or not the bundle tissue contracts is to study its activity, if any, by improved graphic physiological methods.

If Retzer's unique conclusion that the myocardial fibers are replenished from the sinoventricular bundle is correct, it is very difficult to reconcile this interpretation with the facts disclosed by pathological and experimental data. Keith and Miller ('06) reported a case of syphilis of the heart where the superior

vena-cava region of the atrium, the coronary sinus, and the atrioventricular node had been destroyed about thirteen years before death. “. . . the muscular connection between auricle and ventricle had been destroyed several years (probably 13) before death, yet the complete section of the bundle had not resulted in any visible atrophy beyond the point of destruction. Were this peculiar system, . . . only a conducting pathway for the auricular stimuli into the ventricle, one would expect degeneration in the system of fibers beyond the point of section, for it may be taken as an axiom in biology that abrogation of function is invariably followed by atrophy.” This heart showed no signs of failure or patent symptoms of cardiac insufficiency once the patient had recovered from the symptoms incident to the onset of his bradycardia (about forty-two beats per minute) until his death twelve years later as a result of acute peritonitis. If the heart muscle does constantly receive new fibers from the sinoventricular bundle, it is reasonable to expect that there would have been more pronounced symptoms of cardiac insufficiency after the relatively long time that the ventricles had no connection with the sinus region. Furthermore, if there were a constant genetic progress of cells from the sinus to the ventricular myocardium, there would certainly have been marked changes in the myocardium, which were absent in this heart; and especially would there have been profound changes in the part of the conduction system below the lesion. Neither atrophic nor hypertrophic changes are mentioned occurring in the ventricles, nor is either change shown in the sketches accompanying the report. If there were no such changes resulting from the above-described lesion, such would be indirect evidence that the number of cardiac muscle fibers in the adult was the same as the number laid down at birth as stated by MacCallum ('98) and direct evidence against a replenishment of the myocardium from the sinoventricular bundle; for there was no atrophy in the part of the sinoventricular bundle below the lesion. Retzer himself was unable to find any histologic evidence of a degeneration of myocardium in many normal hearts examined.

Keith and Flack ('06) confirmed Tawara ('06) in his finding that this system neither enlarges with cardiac hypertrophy nor diminishes in size with atrophy. Beck and Stokes ('08) and Cohn and Lewis ('12), record the same findings as Keith and Miller as regards failure of the bundle to atrophy beyond a lesion. Erlanger and Blackman ('10) find that this system does not regenerate after its experimental destruction.

Through the kindness of Doctor Erlanger, we have had the opportunity of examining the sections of the ventricular septum of dog no. 8 described in his article to which we have just referred. There is no abnormality above the scar, no regeneration through or around the scar, and no atrophy below the scar. The atrio-ventricular bundle was almost but not quite completely crushed, as shown by the sections. It would seem that conditions here were favorable for at least a partial regeneration, but none occurred. This dog lived for three months and had complete heart-block up to the fifteenth day. From the fifteenth to the forty-second day the block was incomplete. Thereafter the block again became complete and remained so until the death of the dog. Erlanger and Blackman believe that this variable condition may be accounted for by the subsidence of inflammatory processes so that about the fifteenth day physiological connection was again partially established, but that on about the forty-second day the contraction of the scar tissue again broke the partial physiological continuity of the bundle, and again caused complete heart-block.

As regards the regeneration of the sinoventricular bundle, all the evidence shows that it does not regenerate after injury. This fact is also true of all cardiac musculature. Erlanger ('08) crushed the musculature of the atrial appendix in a carefully controlled and aseptic experiment. He found that there was no attempt at regeneration on the part of the myocardial fibers, but that nerve fibers readily regenerated across the contused area. Inasmuch as the atrioventricular bundle is known to be as much a part of the primitive heart as is the myocardium itself (Erlanger, '12) we would hardly expect any difference in this regard between them.

SUMMARY

1. The sinoventricular bundle is not embryonic muscle tissue. It would appear to be a specially differentiated tissue with the definite function of conduction of the impulse to heart beat.

2. There is no adequate histologic evidence in support of the suggestion that worn-out myocardium is replaced from the sinoventricular bundle.

3. Whether or not the sinoventricular bundle has retained the fundamental property of contractility is not to be determined by histological study; it must be determined by improved graphic physiological methods.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Drawing of a section of the sinus node taken parallel to the long axis of the right atrium. The nuclei are large, oval, or elongated, with a random distribution of fine chromatin granules and several nucleoli. Some cells are binucleated. The cytoplasm shows large perinuclear spaces. Striated myofibrils are distributed around the periphery of the cells. $\times 780$.

2 Drawing of a section of the atrioventricular node taken parallel to the long axis of the heart. Description same as in figure 1.

3 Drawing of a section of a false tendon cut at right angles to the long axis, showing: (a) The sinoventricular bundle tissue with the characteristic large oval or elongated nuclei. Many of the cells are binucleated, and the nuclei are closely adjacent to each other. The cytoplasm is pale and contains fine myofibrils in the cell peripheries. (b) Endocardium and connective-tissue sheath. $\times 780$.

4 Drawing of Purkinje fibers (a) in cross-section, showing relative differentiation as compared with adjacent ventricular myocardium (b). The nuclei are larger, paler, and more nearly oval. The perinuclear spaces are much larger than in the myocardium. The myofibrils are fewer, finer, and paler, and placed more peripherally than in the myocardial fibers. (c) Endocardium. $\times 780$.

5 Purkinje fibers in oblique section, showing gradual transition (a) into myocardial fibers (b). The nuclei gradually change in character from the typical Purkinje nuclei to the typical myocardial nuclei. The myofibrils gradually converge and pass insensibly into the myocardial fibers. $\times 780$.

All drawings are from hematoxylin and eosin preparations.