CONDUCTING TISSUES IN VERTEBRATE HEARTS

A HISTOLOGICAL STUDY

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Maurice B. Allen

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CONDUCTING TISSUES IN VERTEBRATE HEARTS

ABSTRACT

The hearts of representatives of five vertebrate classes (Pisces, Amphibia, Reptilia, Aves, Mammalia) were stained with hematoxylin and eosin, Masson's trichrome, Best's carmine, toluidine blue, and 2% silver nitrate. Serial sections were made of the hearts of several specimen.

A connective band of muscle tissue extending from atrium to ventricle was observed in all specimen studied. The degree to which this connecting muscle tissue was differentiated from the atrial and ventricular myocardium varied with the species. Purkinje fibers were observed in the chicken and some species of mammals. In such genera as Rattus, Mus, and Canis there were no typical Purkinje fibers as described for the genera Bos and Ovis. Comparisons as to types of specialized cells in these genera are given with illustrations. Species variations of the bundle of His in mammals are discussed.

A large nerve trunk, not previously mentioned in the literature, was observed in the ventricles of the spade fish (Chaetodipterus faber). Further investigation is needed to determine whether this nerve trunk is characteristic of all advanced Teleostei. A rich ventricular nerve supply with perikarya plus fibers was found to be characteristic of the

alligator ventricle, and of the ventricles of all the mammals studied. No perikarya were observed in the turtle ventricle, but there were many nerve fibers. The majority of the ventricular nerve fibers was of the non-medullated type in all of the species studied, although a few medullated fibers were also present.

A biochemical analysis of the phospholipids of the specialized tissues of the S-A node and His bundle, as compared to the non-specialized tissues of atrium and ventricle, revealed the former to be much richer in phospholipids. This indicates the order of physiological activity of the heart tissues. Percentages are given for each type of tissue.

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A HISTOLOGICAL STUDY

Objective

A tremendous amount of work has been done in the study of vertebrate hearts, especially the mammalian group. However, the studies are not complete. Some groups have been investigated by many workers, while others have been almost completely neglected. In the mammalian group, there is still considerable controversy concerning the conductive pathways. In addition, in recent years, there has been a growing interest in re-examination of the theories concerning the conductive pathways by such men as Glomset (1940, 1945, 1948, 1952) and Field (1950, 1951), in view of their discoveries of a much more extensive intrinsic system of nerves in the atrio-ventricular bundle and in the remainder of the ventricle. For these reasons, it was believed that a comparative survey of representatives of each class of the Subphylum Vertebrata would give a clearer conception of the structures believed to originate the heart beat and conduct the impulse to the remainder of the myocardial tissue in such a manner as to insure synchronization.

BIOCHEMICAL EXPERIMENT

Objective

The differential staining of the conductive tissues in the mammalian heart, especially the sheep and cow, seems to indicate that there is a difference in their chemical makeup. Since the essential lipids are connected with diversified activity (Bloor, 1930), it was thought that a comparison of the lipid content of the tissue of the conductive pathway with the lipids of the remainder of the myocardium would be of some value. Though there have been many analyses of the cardiac tissue, they appear to have been of the total ventricle only. The methods used and the results obtained are included at the end of the histological survey.

TABLE I - METHODS

Animal Classes	Method of Death	Fixative	Embedding	Stains
Pisces:				
2 Sharks (Species unknown)	Unknown	Formalin	Paraffin	1
Teleosts:				
5 Spade Fish (Chaetodipterus faber)	Head Blow	70% Alc.	Celloidin	1-5
2 Sand Trout (Cynoscion arenarius)	" "	" "	"	"
Amphibia:				
12 Frogs (Rana pipiens)	Pithing	70% Alc.	Celloidin	1-5
Reptilia:				
6 Turtles (Pseudemys scripta)	3-ether injection;	70% Alc	Celloidin	1-5
	3-formalin injection	A-F-A mixture	Celloidin	1-5
4 Alligators (Alligator mississippiensis)	Sodium Nembutal	70% Alc.	Paraffin	1-5
Aves:				
6 Chickens (Gallus domesticus)	Decapitation	70% Alc.	Celloidin	1-5
Mammalia:				
6 Mice (Mus domesticus)	Head Blow	(3 Formalin	Paraffin	1-5
		(3 Bouins	"	"
6 White Rats (Rattus rattus)	Head Blow	(3 Formalin	"	"
		(3 Bouins	"	1-5
6 Dogs (Canis familiaris)	Sodium Nembutal	A-F-A 70% Alc.	Celloidin	1-5
12 Cows (Bos Taurus)	Head Blow	A-F-A 70% Alc.	Paraffin	1-5
			Celloidin	"
1 Sheep (Ovis aries)	Head Blow	Formalin	Paraffin	1

Stains: 1)Harris hematoxylin & eosin; 2) Masson's trichrome; 3) Best's carmine; 4) 2% silver nitrate; 5) Toluidine blue.

METHODS

For convenience, the observations resulting from the histological study, and the observations of previous workers in this field have been discussed together for each class of animals. Occasionally where it is necessary to illustrate a point in the histology, reference has been made to certain physiological experiments. The methods used in preparation of tissue for this study have been listed in chart form (Table I). Some explanations of the chart are necessary.

Several different staining techniques were used: 1) Hematoxylin and eosin for routine survey work. 2) Masson's trichrome to demonstrate connective tissue. 3) Toluidine blue for nervous tissue. 4) Cajal's silver nitrate modification for nervous tissue (fide Gray, 1954). 5) Best's carmine for demonstrating glycogen.

Two types of toluidine blue techniques were used. The Krajian process (1940) was used for tissue already embedded and sectioned. The Krajian technique was modified with very satisfactory results by substituting bergamot oil for cajuput oil. The Landau technique (fide Gray, 1954) was used as a bulk staining process.

In general, the time recommended for bulk staining resulted in overstained tissues, which necessitated destaining. For toluidine blue, 70% ethanol was found to be effective except in cases of densely overstained material. In this event, a mixture of equal parts of bergamot oil, xylene, and creosote mixed with an amount of absolute ethanol equal to the total of the three other ingredients was found to be effective and rapid.

The silver nitrate sections were very difficult to destain; the only effective agent was an equal mixture of potassium ferrocyanide and sodium hyposulfite (Krajian, 1940).

The Best carmine method for glycogen determination as a test for Purkinje cells was used on the chicken heart. Two methods were used as checks upon each other. The Krajian modification required 5% benzoic acid as a mordant. The Gomori (1953) modification was an alkaline method, which made use of a stock carmine solution diluted with 15 c.c. of NaOH and 15 c.c. absolute ethanol for every 10 c.c. of the stock solution.

The chickens were purchased at the age of two weeks, fed on Purina Growth Mash for two weeks, and killed by decapitation. The hearts were removed even before they had ceased to beat. They were fixed in 70% ethanol and embedded in celloidin.

OBSERVATIONS AND DISCUSSION

CLASS PISCES

Histology of Conductive Tissues

Relatively little work has been done on the hearts of fishes. The most comprehensive work is that of McWilliams (1885), on both the structure and physiology of the heart of the teleost eel. Keith (1907) dissected and sectioned the hearts of three fishes; eel, shark, and salmon. McQueen (1913) studied the Elasmobranchs. Skramlik (1930) and Bielig (1930), working independently, did an excellent series of physiological studies, in which they established the existence of three different types of hearts among the fishes as to the origin and conduction of the beat (Fig.1). Type A, found in the primitive teleosts, showed the primary origin of the impulse to be located in the sinus venosus, a second slightly less sensitive conductive center located in the auricular canal at the junction of the sinus and atrium, and a third much less sensitive center at the mitral orifice on the atrial side. Type B hearts, found in the selachians, have the primary origin in the sinus and a secondary center of electronegativity at the mitral orifice. Type C hearts, found in the majority of teleosts, have the primary center in the atrium at the sinus junction and a secondary center at the mitral orifice.

In consideration of these data, the hearts of two sharks (species unknown), two sand trout (Cynoscion arenarius), and five spade fish (Chaetodipterus faber) were examined carefully for signs of structural differences which would account for the physiological phenomena. In both the sharks and teleosts the sinus wall is very thin; the auricular wall is slightly thicker, and the ventricular wall is the thickest. The cells of the sinus are rather poorly developed compared to the cells of the ventricle. The sinus cells are very narrow with a few fibrils located mostly in the peripheral area, with cells grouped in very thin bundles. These bundles extend in varying directions separated by connective tissue, so as to present a reticular appearance. The amount of connective tissue is impressive in comparison with the amount found in the auricles of higher animals. The auricular cells are intermediate between the sinus cells and the ventricular cells in both size and clarity of striations. This corresponds to McWilliams' (1885) observations in the eel.

The most striking difference between the fish heart and that of the higher animals is the comparative isolation of the auricle from the ventricle of the fish heart by means of the auricular canal. McWilliams (1885) found this to be

true in the eel and it has been confirmed in the animals used in this study.

The connection from the sinus to the ventricle is direct. The posterior wall of the sinus is extended and forms the basal wall of the auricular canal. This band of muscle quite visibly terminates at the mitral orifice and forms a complete ring, with the fibers arranged in a circular direction. The auricle in the fish is connected to the ventricle by only one wall.* The other auricular wall is attached to the sinus venosus. The main body of the auricle is separated from the ventricle by the auricular canal, except for the one wall connection. According to the findings of McWilliams (1885) concerning the teleost eel, the auricular connection to the ventricle may be either clamped off or severed and the heart will continue to beat at the normal rhythm. On the other hand, his experiments showed that the auricular tissue was also capable of conducting the impulse, for if the basal wall was severed, but the connection between sinus and auricle was left intact, the impulse would still reach the ventricle, although the time interval was longer. Thus we see in the fish a double pathway from the sinus to the ventricle: 1) through the differentiated tissue of the basal wall; 2) through the undifferentiated auri-

*It should be remembered that the auricle of the fish heart is all of the atrium except the auricular canal.

cular myocardium. In the codfish (Gadus virens), Bielig (1930) found "the sinus nodal tissue is in the form of a muscular sphincter at the S.A. junction with two lines of tissue radiating into the atrial wall" (translation).

The only discernible structural difference which might account for the origin of the beat in the sinus proper in the shark, but in the auricle of the teleosts, is the comparatively great reduction in the size of the sinus venosus in the majority of the teleosts. Although the sinus size varies greatly in the teleosts, it is one-third or less the size of the auricle in any one species of fish. It is reduced to considerably less than one-third the size of the auricle in the spade fish, but the studies of Bielig (1930) and Skramlik (1930) which included 25 species of teleosts indicate considerable size variation.

The beat of the Type C heart originates on the auricular side of the junction of the auricular canal and the sinus. Although there is a difference in the site of the origin of the beat, there is no difference in the type of tissue in which it originates, for the basal wall of the auricular canal is an extension of the sinus type tissue. Actually, the only change in the origin of the beat is to restrict it to a certain part of the sinus tissue. The change is from the ostial part of the sinus (Type A), as

noted by McWilliams (1885) in the eel, to the entire sinus (Type B), as noted by Bielig (1930) in the shark, and then to the entrance of the canal (Type C) in the heart of the advanced teleosts, as mentioned by both Bielig (1930) and Skramlik (1930).

As has been stated above, the mitral orifice is ringed with what is considered an extension of sinus tissue, although at this point the cells are enlarged (Fig. 2). Extending at right angles from this ring to the ventricle are what appear in section as finger-like-projections of the same type of tissue. McWilliams (1885) observed these in the eel and the present observations on the spade fish and sand trout correspond. These projections may form the conductive paths for the impulse to the ventricular tissue. They are found around the entire ring.

The ventricular musculature is histologically separated into two distinct parts: an outer compact layer and an inner spongy layer (Fig. 3). There is no morphological difference in the individual cells of the two areas. The difference lies in the compactness and orientation of the bundles of fibers. The outer compact layer is thin in comparison to the inner spongy layer. The thickness of the compact layer varies in different species, but there are always at least two layers of muscle fibers--an outer longi-

tudinal and inner circular. The spongy area has the bundles of muscle fibers extending in so many directions as to present a reticular aspect in section. The connective tissue is much more abundant in the spongy layer. In the species examined, the compact layer of the teleost heart is relatively thicker than the compact layer of the shark heart. It is interesting to note that in bulk preparations the compact layer stains several shades lighter than the spongy layer. The contrast is particularly striking with toluidine blue (Fig. 3). The extensions of the mitral ring make their connections with musculature of the spongy layer.

McWilliams claimed a distinct differentiation of the basal wall of the canal in the ring area for the eel. This differentiation was not very pronounced in the shark and spade fish (Fig. 2).

McWilliams (1885), in his work on the eel heart, did extensive experiments on the innervation by the vagus nerve. He concluded that the vagus nerve has no direct control over the fish ventricle, since, when the heart was in vagal inhibition, a direct stimulation, even a mild one, would produce ventricular contraction without affecting the inhibited state of the auricle and sinus. On the other hand, direct stimulation of the auricle or the sinus during vagal

inhibition produced no effect. This would indicate an absence of nerve fibers in the ventricular area if current views concerning nerve participation in the heart beat are correct. There are no macroscopically discernible nerve connections to the fish heart for the sympathetic chain as there are in the hearts of the higher vertebrates (McWilliams, 1885 and Hyman, 1941). Yet there are definitely nerve fibers in the ventricle of the spade fish. There are thin fibers in the spongy layer and a large nerve trunk as well as individual nerve fibers in the compact layer of the heart (Fig. 3). The abundance of nervous tissue in the compact layer of the heart of the spade fish was striking. None was observed in the shark heart.

CLASS AMPHIBIA

Histology of Conductive Tissues

Though a great deal of work has been done on the hearts of the amphibia, most of it is physiological in nature. Only two surveys bear any great relation to this particular problem. Noble (1931) has the best treatment for general orientation. The only work on connecting or conducting pathways in the amphibia was done by Keith on the frog and by Davies(1941) on the heart of the salamander. In this study, the frog, Rana pipiens, was used.

In gross structure the frog heart is greatly advanced over that of the fish, since it has two atria and other modifications to accommodate a double circulation. Histologically the advance is very irregular, and there seems to be almost a regression in some aspects. The origin of the beat is in the sinus, a fairly large structure located on the dorsal area of the heart and emptying into the right auricle. The entire sinus, according to Skramlik (1930), participates in the origin of the beat as the pacemaker of the heart. The musculature of the sinus, like that of the sinus of the fishes, is thin. The individual fibers, or cells, are somewhat larger and better developed than in the fishes. The same relationship as to size and myofibrils in the individual cells ob-

tains in the frog as in the fish, i.e., the cells of the ventricle are larger than the cells of the auricle, which are in turn larger than the cells of the sinus.

McWilliams (1885) mentions, in his work on the eel, that the eel was unlike the frog in that in the teleost eel the sinus joined directly with the ventricle by a projection of tissue known as the basal wall of the auricular canal, whereas in the frog the sinus joined to the atrium. In this investigation, it was found that the lower wall of the right atrium differed from the other parts of the atrium in paleness of staining and in having fewer myofibrils. This would indicate that the basal wall of the auricle had its origin from sinus tissue, even though the greater development of the atrium tends to mask the sinus origin of the basal wall. Keith's (1907) findings seem to substantiate this view, although the number of frogs he used in his study was limited to three. Davies' (1941) findings on the heart of the salamander are substantially in agreement.

As in the fish, there is a definite circular arrangement of muscle fibers at the mitral orifice with atrial tissue projecting into the ventricular musculature. The ring is somewhat thicker just beneath the septal wall and longitudinal projection appears slightly thicker in this area. This tends to substantiate the observations of Keith

(1907), though the difference in thickness is barely discernible in the specimen used in this study.

These finger-like projections of the ring merge with the spongy tissue of the ventricle. The myocardium of the ventricle of the frog is all spongy tissue with bands of fibers extending in many directions (Figs. 4 & 5). There is no compact outer layer of the myocardium as in the fishes.

Nerves

There are fewer large nerve fibers in the ventricular tissue of the frog than in the tissue of the fish, although the auricular tissue is quite rich in nerve fibers and ganglia. There are, however, fibers from the sympathetic system as well as from the para-sympathetic (McWilliams, 1885). It is interesting to note that, according to McWilliams, the frog heart will react to a direct stimulation in any part while under vagal inhibition, perhaps due to the presence of opposing sympathetic nerve fibers; whereas in the teleost eel only the ventricle would react to direct stimulation under like circumstances.

CLASS REPTILIA

Histology of Conductive Tissues

In the reptiles there is more than one type of heart, the major difference being in gross anatomy rather than in the histological structure of the heart. One type, represented in this study by the turtle, has an incomplete ventricular septum. The second type, represented by the alligator, has a complete ventricular septum. The turtle has a sinus that is much reduced, and the alligator also has a sinus, which being incorporated into the right atrial wall is not visible externally.

The histological aspect in the reptiles has been neglected because there are no striking contrasts in tissue as there are in the mammalian group. Most of the histological work has been done as a necessary adjunct to physiological experiments to clarify certain issues. Among those who have done physiological experiments with some mention of anatomical or histological data are Gaskell (1881) on the tortoise, Skramlik (1930) and Harris (1941) on the turtle. Keith (1907) and Laurens (1913), using the turtle and lizard, approached the problem from the structural point of view. Davies (1941) considered the conductive pathway of the alligator.

The structure of the conductive tissues of the reptile

is similar in many respects to that of the amphibia and pisces, but there are significant differences. One difference has already been mentioned, the incorporation of the sinus into the auricular wall in the alligator.

In the reptile, as in the pisces and amphibia, the mitral orifice is ringed by a muscle band of cells which are distinct because of their circular direction. Keith (1907) maintained that these are a continuation of the sinus cells of the basal wall and that they show a distinct differentiation. Gaskell (1881) spoke of the cells of the basal wall as having a distinctive appearance. In the present observations on the turtle (Pseudemys scripta) the basal wall cells were easy to distinguish, were definitely different in their staining characteristics (paler), but were no larger than the other auricular cells. Keith found the basal wall cells of the turtle to be larger than those of the auricular myocardium. The canalis auricularis is not as distinct in the reptiles as it is in the fish. The ring extends around the opening of both A-V valves. Skramlik (1930) reported in his physiological study of the heart beat of Testuda gracia that the path of the excitation impulse is confined to the ventral side of the ring. There is no differentiation of the cells of this part of the ring. There is, however, a thickening of the ring in this area, particu-

TABLE II

CELL SIZES IN VARIOUS PARTS OF THE HEART
All figures are an average of ten cells measured in micra across the diameter.

Animal	Sinus or S-A node	Auricle	Mitral Orifice or A-V node	Connecting Muscle or A-V bundle	Ventricle
Shark	6.2	10.2	8.1	8.3	14.9
Frog	6.3	11.1	8.2	8.4	15.1
Turtle	4.3	6.2	6.1	6.2	9.4
Alligator	6.1	9.2	8.3	8.4	13.1
Cow	13.5	10.3	11.2	15.9	13.9
Mouse	6.1	5.9	6.2	7.5	7.3
Rat	6.9	6.4	7.1	7.8	7.4

larly near the auricular septum. Keith (1907) reported this fact for the lizard, and the present observations confirm it for the turtle and alligator.

There is nothing in the turtle or the alligator heart resembling the bundle of His or the Purkinje cells of the mammals. The projection of the mitral ring tissue extends from the entire circumference of the ring, although it is thicker in the septal area. The fibers of the projection equal the auricular fibers and are smaller and less eosinophilic than the ventricular fibers into which they extend. (Fig. 6).

The fibers of the auricle and the ventricle are, in general, larger than those of mammals of corresponding body size. (Table II-opposite page).

There appears to be as much differentiation between the ring and projection cells and the other ventricular musculature of the reptile as there is between the nodal and surrounding tissues in such mammals as the rat.

Nerves

In none of the previous studies has much attention been paid to the nerves of the ventricle of either the turtle or the alligator. Mention of the rich nerve supply of the auricles, the sinus ganglion, and the A-V junction ganglion has been made by all the authors from Remak (1838) and

Bidder (1842) through Keith (1907) and Davies (1941). The present study confirmed these findings, and in addition, found a very rich nerve supply in the ventricle of both the turtle and alligator.

The ventricular nerves of the turtle appear primarily as several large fibers extending downward from the atrial area with multitudinous branches (Fig. 7). No myelinated fibers were observed. It is impossible to distinguish histologically between the fibers of the sympathetic accelerator nerves and the vagal inhibitor nerves even in the auricles, since in the heart the majority of the vagal fibers are non-myelinated.

A different situation was observed in the heart of the alligator. In addition to the fibers which extended longitudinally from the atrium, there were numerous fibers which pursued an oblique or circular course (Fig. 8). These were more numerous than the longitudinal type. They lie between the cells either singly, in pairs, or in groups of four or five fibers adjacent to each other. No large nerve trunks were observed. In the upper portion of the ventricle several perikarya were observed with an oil immersion objective. In contrast to the major type of atrial perikaryon which is stellate, a round, light staining type predominated in the ventricle. In the absence of physio-

logical experiments, it is impossible to draw any conclusions from these data except that the nerve supply to the ventricles is far richer and, in the alligator, more diversified than has been previously mentioned in the literature.

CLASS AVES

Histology of Conductive Tissues

There is some controversy concerning the conductive tissues of the birds. Davies (1930) made a concise summary of the varying views on the system in the bird as follows: 1) It consists only of subendothelial and perivascular network of Purkinje fibers in the auricle and ventricle (Hoffman, 1902, Tawara, 1906). 2) It has no sino-atrial and atrio-ventricular nodes (Keith, 1907, Mackenzie and Robertson, 1910). 3) It has an atrio-ventricular bundle on the right side only (Flack, 1911, Mackenzie and Robertson, 1910, Kulb 1913). 4) It has an S-A node, an A-V node, and a bifurcating bundle (Mangold and Kato, 1915, Clark, 1927, Ohmori, 1928).

In his study, Davies (1930) observed an atrio-ventricular node and a bifurcating bundle with a recurring branch which extends to the root of the aorta. He also found a ring of Purkinje fibers extending around the right atrio-ventricular ofifice and connecting with the ventricular and auricular musculature through the muscular valve. In addition he found two subendothelial Purkinje networks which do not connect with each other in the auricle and ventricles. He further observed that there is no connective tissue sheath separating the bundle from the ordinary myocardium.

In his work, he used the pigeon, swan, ostrich, stork, and penguin.

The present study has been limited to the chicken (Gallus domesticus). A subendothelial Purkinje system was observed quite clearly in the atria and ventricles, and some possible Purkinje cells were seen in the region of the right atrio-ventricular valve. No definite sino-atrial, atrio-ventricular nodes, or bifurcating bundle with its branch to the aorta was found. This may be due to difference in technique. Davies (1930) stated that being aware of the difficulty in tracing the conductive system of the bird, he fed his pigeons (the most emphasized bird of his study) on a special diet for several weeks before killing them. He then used Bauer's and Best's method of glycogen staining, tracing the various parts of the Purkinje paths through the intensity of the glycogen stains. No specially enriched food was used for the birds of the present study. They were fed on Purina growth mash and killed at the age of four weeks. By the use of hematoxylin and eosin stains, which will reveal specialized fibers in the beef heart quite readily, no nodes or bundle were revealed in the chicken heart.

The glycogen techniques used in the present study were the Krajian (1940) and Gomori (1953) modifications of the

Best carmine method. Both revealed a greater concentration of glycogen in the ventricles than in the atria, and a far greater concentration in the left ventricle than in the right. There was nothing in the distribution of the glycogen particles to indicate a pathway; nor could there be seen any greater concentration of glycogen in the subendothelial Purkinje fibers than in some of the cells of the non-specialized myocardium.

It seems appropriate at this point to discuss the major ideas concerning the reliability of the glycogen method in tracing conductive tissues. It has been applied to both mammals and birds, but more often to mammals. Aschoff and Nagayo (1908) did much to establish the theory that Purkinje fibers could be located by staining for glycogen. Lewis (1921) accepted the efficacy of the glycogen method completely. He set up a table correlating the conductivity and the glycogen content of heart tissues. Buadze and Wertheimer (1928), however, found seven times as much glycogen in the ordinary myocardium as in the Purkinje fibers of the horse. Davies (1930) showed the fallibility of the glycogen staining technique as a means of locating Purkinje fibers by his insistence on a specially enriched carbohydrate diet. Glomset (1945) stated that he could find no relation between conductivity and glycogen content.

Field (1950), working with the embryonic sheep heart, could find no more glycogen in the Purkinje fibers than in many other myocardial cells.

Thus it can be readily seen that the results of this glycogen study are in agreement with the conclusion of the majority that the glycogen staining technique is not one to depend upon in tracing Purkinje fibers.

Nerves

A rich supply of non-myelinated nerves was observed in the auricles, which is in accordance with the studies of other workers (Hofmann 1902, Keith 1907, Flack 1911, Davies 1930).

None of the above workers except Davies, who found them negligible in quantity, discussed nerve fibers in the ventricle. The toluidine technique showed fewer and finer nerve fibers in the ventricle of the bird than were found in the turtle and alligator hearts. The silver nitrate method demonstrated many very fine nerve fibers forming a network about the cells of the ventricular myocardium.

CLASS MAMMALIA

Histology of Conductive Tissues

Because so very much work has been done on the mammalian heart and so many controversies exist concerning its structure, it is believed that the best method of presentation will be to list the major ideas and to include in parentheses the authors favoring each idea.

1. A Sino-atrial node of specialized tissue (Keith 1907, Davies 1935, 1942, 1946, 1952, Field 1950).
   No Sino-atrial node of specialized tissue (Glomset 1940, 1945, 1948, Prakash 1954).
2. Several pathways from auricles to ventricle (Kent 1893, Glomset 1942-50, Field 1950).
   Only one pathway through heart (His 1893, Tawara 1906, Keith 1907, Davies 1952, King 1954, Prakash 1954).
3. Bundle of His of specialized cells bifurcates into right and left branches, arborizes and terminates in subendothelial Purkinje system (His 1893, Tawara 1906, Keith 1907, Davies 1935, 1952, Truex & Copenhaver 1947, Field 1950).
   Bundle of His does not bifurcate in certain species (Glomset 1940, 1945, 1952).
4. Conductive pathway is neomorphic (Davies et al 1945).
   Conductive pathway is primitive retention (Keith 1907,

Field (1950).

5. Importance of nerves underestimated (Todd 1919, Agduhr & Stenstrom 1928, Wahlin 1935, Davies 1935 Glomset 1940-52, Field 1950, 1951).
   Importance of nerves overestimated (Gaskell 1881, Keith 1907, 1910, Mackenzie 1910, Flack 1911).

In this survey the mouse (Mus domesticus), rat (Rattus rattus), dog (Canis familiaris), sheep (Ovis aries), and cow (Bos taurus) were used. Efforts were made to determine the structure and position of the sino-atrial node, the atrio-ventricular node, the bundle of His, and the sub-endothelial Purkinje system.

SINO-ATRIAL NODE

Observations

A well demarcated S-A node of the typical fibers as originally described by Purkinje (1845) was observed in both sheep and beef hearts.

The node of the beef heart has a central compact area made up of strands of fibers which are coiled about each other, presenting a reticulate mass (Fig. 9). Strands of fibers project from the mass in various directions merging with the auricular myocardium. The node of the sheep is more compact and somewhat fan shaped. The fibers of the

nodes of both sheep and cow hearts are smaller than those of the atrio-ventricular node. They have the characteristic pale staining quality and the peripheral fibrillation, but unlike the typical Purkinje fibers, they are mononucleate.

The S-A node of the rat and mouse hearts is ovoid in shape as seen under low magnification. Under high power the fibers of the node differ very little from the cells of the myocardium in size, shape, or paleness of staining. Except for the location of the node under low power, it would be difficult to make any distinction between the nodal and myocardial tissue.

In the dog the S-A node is difficult to distinguish. The fibers of the node are somewhat thinner than those of the remainder of the auricular myocardium.

Discussion

The above observations are in agreement with the findings of most workers in this field.

Keith and Flack (1907) discovered the sino-atrial node. Using the kangaroo, mole, whale, rat, kitten, ram, pig, and horse, Keith and Flack found the S-A node to be most prominent in the mole. They concluded that the S-A node was a remnant of the sinus venosus of the embryo and corresponded to the sinus venosus of the lower vertebrates. They considered the node a retention of primitive tissue both as to

its function and morphology.

Most subsequent workers have found a node of special tissue in mammals, located either at the base of the superior vena cava or in the area between the inferior and superior vena cavae. The exact position varies with the species.

Glomset (1940,1945,1948,1952) disagreed with the conception of a sino-atrial node. In his research on the dog, he found an S-A node. He traced its ramifications and found it to be a continuation of a muscle band stretching across the dorsal auricular walls (Brachman's Bundle). He found no specialized tissue in the S-A node. The fibers of this area were slightly smaller than those of the auricular myocardium; otherwise there was no discernible difference. He also found patches of fibers of similar nature in other parts of the auricles. He questioned the role of an S-A node as the pacemaker in the heart of the dog. He did find, however, a well defined S-A node composed of typical Purkinje fibers in the beef heart.

In this study the hearts of rats and mice, as well as dogs, were examined, and found to be very much as Glomset described them. Under low power the S-A node is easily visible in the mouse and rat heart (less so in the dog) as an ovoid muscular mass. Under high power, however, the distinction between cells of the S-A node and the cells of the

surrounding tissues is difficult to determine even in the mouse and rat. The S-A fibers do seem somewhat larger, with more light staining sarcoplasm, and with myofibrils more peripheral than in the fibers of the surrounding myocardium. These distinctions are almost non-existent in the dog.

Prakash (1954) found a sinus venosus rather than a sino-atrial node in the rat heart, although this claim is highly questionable. Nothing in the literature or the observations of this study confirms his findings.

The conclusion reached from the observation of tissues and from the literary review is that a sino-atrial node of greater or less cell differentiation appears to exist in all mammals. Hoff (1945) has shown that even if Glomset's observations concerning the S-A node and Brachman's Bundle are correct, the fact that the node is a part of a larger muscle bundle does not necessarily invalidate it as a node either structurally or functionally.

ATRIO-VENTRICULAR NODE & BUNDLE OF HIS

Observations

An atrio-ventricular node was observed in the heart of each mammal studied (mouse, rat, dog, sheep, cow).

Typical Purkinje fibers were observed only in the A-V node of the cow and sheep hearts (Fig.10).

Fibers of the mouse, rat and dog atrio-ventricular nodes are not typical Purkinje fibers. In the mouse and rat, the A-V nodal fibers are somewhat larger, with more pale-staining centrally located sarcoplasm than the fibers of the adjacent tissue. In the dog, the fibers of the node are the same size or even smaller than those of the surrounding myocardium.

The bundle of His in the beef and sheep hearts descends from the A-V node through the ventricular septum. After a short distance, it bifurcates, sending a branch to each ventricle. The fibers are typical Purkinje fibers; i.e., they are large, pale staining, peripherally fibrillated with a large amount of central sarcoplasm.

The bundle of His in the mouse, rat, and dog is very difficult to trace microscopically, because the fibers are not clearly differentiated from those of the ventricular myocardium. At its beginning, the compactness of the bundle is easy to discern, but any bifurcation is difficult to see. Even serial sections failed to reveal subdivisions of the bundle.

Muscular connections other than the bundle of His can be seen between the right atrium and the right ventricle of the rat, mouse, and dog.

Discussion

The muscular connecting paths between the right atrium and right ventricle were first observed in mammals by Kent (1893). He used the rat in his study and reported the existence of several muscular connections.

His announced the discovery of the main muscular path in 1893 and again in 1903 (Translation of the His works by Gardner and Bast 1949). Tawara (1906) announced the discovery of the A-V node.

The several pathways previously mentioned by Kent were ignored, and most research and publication were concentrated on the bundle of His.

Glomset (1940, 1945) revived interest in these alternate paths with his observations on the dog heart. Field (1950) corroborated the presence of alternate pathways in his work on the sheep heart. The present study confirmed the existence of alternate A-V muscular connections in the mouse, rat, and dog.

Baird and Robb (1950) denied the existence of the alternate pathways in the dog and claimed to have traced a bifurcating bundle of His to many fine branchings.

Davies (1946) believed the His bundle and branches to be a neomorphic development in mammals rather than a retention

of primitive tissue. He based his ideas on the histological sectioning of embryonic wallabies. Field (1950), basing his work on the embryonic sheep heart, concurred with Keith and Flack (1907) that the bundle of His was a retention of primitive tissue.

On the basis of slides prepared for the present study, there seems to be sufficient similarity in the arrangement and positions of various cardiac muscle fibers in all classes of vertebrates to justify the position that these fibers represent a primitive system.

SUBENDOTHELIAL-PURKINJE SYSTEM

Observations

A subendothelial Purkinje system was seen in the ventricles, but not in the atria, of all the mammals studied. Some possible exceptions were observed in isolated patches of cells in the atria of the dog and rat which might have been Purkinje cells.

The ventricular subendothelial Purkinje system was very well developed in the ungulates used in this study, but very difficult to follow in the rodents and carnivores, because there is so little differentiation between the so-called Purkinje cells and the other myocardial cells in these orders (Fig. 11).

Discussion

Purkinje (1845) described what he called a specialized neuro-muscular cell (a pale staining cell with much central sarcoplasm, peripheral fibrillation, frequently with double nuclei) in the ventricle of the cow and sheep hearts. Since his time there has been a tendency to call any specialized heart muscle fiber believed to have a conductive function a Purkinje cell.

Davies (1946) found the Purkinje system of the horse confined to patches which he considered the remains of a degenerated system. Ter Borg (1948) limited his study to the atria, where he found a well developed Purkinje network in both right and left atria of the horse, pig, sheep, and goat.

Glomset (1945) discovered no Purkinje system or cells in the dog, because he refused to accept as Purkinje those cells which are not typical.

The crux of considerable controversy is the definition of a Purkinje fiber. Should it be limited to the typical cell as described by Purkinje, found in sheep and cattle, or should it include any differentiated cardiac cell of a conductive pathway? (Fig. 12)

Davies (1930-1952) acknowledged the great species vari-

ation in the Purkinje fiber, but maintained that any differentiated muscle cell of a connecting pathway should be accepted as a Purkinje fiber.

The present study confirms the fact that considerable differences exist in the cardiac musculature of several animals. In the rat and mouse, the specialized fibers are only slightly larger than adjacent cells and have somewhat more sarcoplasm centrally located. In the dog, the fibers of the A-V bundle are of the same size or even smaller than the adjacent myocardial cells.

If the interpretation of the Purkinje cell be restricted to the cells described by Purkinje (favored by Glomset), then there is no Purkinje system in many mammalian species. Yet in all those species which possess the doubtful Purkinje fibers, the present work and that of all previous workers, including Glomset, shows that where bundles of these cells exist they stain lighter than the surrounding tissue.

If the looser interpretation of Purkinje fiber, as any part of a cardiac muscular conductive pathway which shows a differentiation be accepted for the mammals (Davies favors this), then it seems that the same interpretation would apply to the similar fibers in lower animals.

The irregularity of the appearance of the Purkinje system among mammals (restricted interpretation) would require some explanation. Assuming the Purkinje fiber to be

a neomorphic development among the mammals, then it is more logical to assume it a mutation common to the ancestoral types, secondarily lost in some species, than to assume that some species developed the mutation while others did not.

Nerves

An excellent review of the literature concerning the nerves of the auricle can be found in the work of Kuntz (1945) and that of Nonidez (1939 & 1949). The present study is limited to the nerves in the ventricular area.

Ventricle

Observations

The majority of the fibers are non-myelinated. Nerves in the bundle of His found in the cow are exceedingly numerous. They extend through the bundle and send branching fibers in several directions. Nerves in the mouse, rat, and dog hearts are mainly in the periphery of the bundle (Fig.13). Cytons of two types were observed in basal area of the ventricular musculature of all the animals studied. Cytons occurred singly or in groups of two or three. There were no large ganglia. The two types of cytons observed were round and stellate.

Discussion

Lawrentjew and Gurwitsch-Lasowskaja (1930) found many

nerve fibers in and about a poorly defined bundle of His in the rat. The fibers of the nerves were mainly non-mylinated, but a few medullated fibers were also seen.

Davies (1935) in writing of the beef heart stated that the nerves were so numerous that the bundle might well be called a neuro-muscular bundle. He found cytons either singly or in clusters covering the basal two-thirds of the wall of the ventricle in great number, becoming fewer in the apical portions. He classified the cytons into two types: 1) Cells of round shape, light staining, which he concluded were afferent in nature. 2) Stellate cells, dark staining, which he believed to be efferent in nature.

Nonidez (1939) stated that it was possible to distinguish the afferent and efferent fibers by the intensity of staining, the afferent staining lighter. It was impossible to distinguish the difference in this study either by the toluidine method or the silver nitrate method. Nonidez used very young or foetal animals. He used the same Cajal Chloral Hydrate silver method used in this study. Either Nonidez' technique was able to produce a finer differentiation of sections or his use of foetal specimens may have been the deciding factor. Field (1950) experienced the same difficulty in making a distinction of afferent and efferent fibers by staining methods that was experienced in this study.

Glomset (1945), who opposed the Myogenic Theory, found nerves in the dog ventricle, but around the bundle rather than in it. The present study agrees except for the presence of some nerve fibers in the bundle.

Field (1951) in his observations of the sheep found many nerves in the bundle, and in addition, many fibers extending in all directions throughout the ventricle itself. He also found many perikarya in the A-V groove and ventricle. Field (1951) was unwilling to contradict the Myogenic Theory, but he did state that he felt that the work of Glomset et al (1945), and his own observations indicated the necessity for a comprehensive reexamination of the heart conductive pathways and their interpretation.

Agdhur and Senstrom (1928) performed an experiment in which they degenerated the muscular fibers of the bundle of His with massive doses of cod liver oil. The heart beat continued very close to normal. The mouse was killed and the bundle examined histologically; the nerve fibers were intact and the muscle fibers of the bundle were degenerated. Wahlin (1935) repeated the experiment and obtained the same results. Maximow and Bloom (1948) stated: "This conduction system is accompanied by many nerves which also play a part in carrying the contractile impulse. The numerous nerves of the heart belong in part to the vagus

nerve and in part to the sympathetic nerves. Some nerve endings in the heart are apparently of the effector type, while other endings are of receptor or sensory character."

Field (1951) questioned the validity of the physiological experiments such as the second Stannius ligature and the Gaskell clamp as proof of the conductivity of the bundle muscle fibers; since the nervous and muscular elements are so closely associated, it is impossible to interrupt one without interrupting the other. Hence such experiments would merely prove the bundle of His the structure of conduction, giving no proof as to which element within that bundle did the conducting. His observations concerning the intimacy of the nervous and muscular elements are substantiated by a long line of preceding workers (Tawara 1906, Biggs 1908, Wilson 1909, Cohn & Trendelberg 1910, Engel 1910, Scaglia 1927, Perry and Rogers 1934, Glomset 1940-52, Nonidez 1939, 1949).

Robb and Kaylor (1945) concluded that greater correlation of physiological and histological experiments was needed. In their examination of the Guinea Pig they found a well marked bundle of His with many clear branchings of the bundle. Although there were many more branches of the bundle in this animal then in many other mammals, the electrocardiogram

was the same as that of animals with few branches. In other words, there was a distinct discrepancy in the physiological and histological interpretations.

A COMPARATIVE STUDY OF PHOSPHOLIPIDS IN THE HEART

Objective

The object of this experiment, as stated in the introduction, was to ascertain if there was a difference in the phospholipid content of the conductive pathway and the auricular and ventricular musculature of the beef heart.

Literature

There is no literature on such a comparison. Bloor, Okey, and Corner (1930) did an analysis for cholesterol and phospholipids of total heart ventricle, in thich they found total essential lipids to be approximately 7% of dry weight. They state: "Cholesterol and phospholipids constitute an integral and constant component of tissues, the amount of which is not essentially altered by extreme changes in the nutritional status of the animal." Neutral fats were found variable. Bloor and Snider (1934) made a study correlating phospholipid content and muscle activity in the heart. It was concluded that the lipid content (cholesterol and phospholipids) was much greater than that of striated muscle and smooth muscle. Bloor (1936) found cholesterol content highest in smooth, second in cardiac, and third in striated muscles. In cardiac muscle he found the phospholipid to cholesterol ratio to be 20 to 10. Phospholipids were the

most abundant lipids of heart tissue and other muscles and cholesterol the least in amount. The amount of phospholipids is related to the activity of the tissue, not the species. Kaucher, Galbraith, Button, and Williams (1943) made a comparative study of beef organs in which they found the heart was exceeded in lecithin only by the liver, kidney, and brain; in cephalin only by the liver and brain; and in sphingomyelin by the lung, kidney, and brain. In total phospholipids the brain was 26.37% of dry weight (total lipids 51.59%). The heart contained 9.83% phospholipids by dry weight (total lipids 16.45%).

Methods

The method used in this study was Bloor's (1926) for analysis of phospholipids of the beef ventricle. The portions used were different, since he used a thousand grams of beef ventricle; otherwise his method was followed exactly. The portions used in this experiment were derived from five beef hearts in one case and seven in the other. 36 and 45.1 grams wet weight of sino-atrial tissue, 58.6 and 50 grams of auricular tissue, 122 and 119 grams of bundle, and 82.5 and 111 grams of ventricular tissue were used. The tissue was obtained from beef hearts removed within 15 minutes of the death of the animals. The various parts of

the hearts were dissected with as great speed as possible. All epicardial adipose tissue was removed. In one instance, the tissue was frozen and pulverized with mortar and pestle. In the other analysis, the tissue was ground immediately by a fine attachment in a meat grinder. No other difference was made in procedure in the two analyses.

The steps followed in Bloor's method are as follows:

I. Extraction

1. Grind meat.
2. Extract with three volumes of 95% ethanol at 35° C for a minimum of two hours.
3. Filter through a Buchner funnel and rinse with ethanol.
4. Extract with hot ethanol three times for a minimum of one hour each.
5. Filter with Buchner funnel and wash with hot ethanol.
6. Extract with ether.

II. Recovery of Lipids

1. Distill off ethanol at 40° C in partial vacuum.
2. Separate in separatory funnel.
3. Wash with ether and extract with ether. Use large volume for best results. Sphingomyelins will be separated here as they are insoluble in ether.

4. Recovery of phospholipids
   a. Evaporate ether to 100 c.c.
   b. Centrifuge with acetone to precipitate cephalin and lecithin.
   c. Dissolve precipitate repeatedly in dry ether (two or three times).
   d. Add insoluble material to sphingomyelins.
   e. Centrifuge to clearness.
   f. Precipitate cephalin by adding an excess of absolute ethanol.
   g. Evaporate the ethanol solution to dryness in a partial vacuum at 40° C.
   h. Dissolve the cephalin in dry ether up to 50 c.c. volume.
   i. Dissolve the lecithin in dry ether up to 50 c.c. volume.
   j. Evaporate the ether in a steam bath.
   k. Complete drying in desiccator of $CaCl_2$.
   l. Weigh.

III. Tests for Substances
   1. Solubility in ethanol, ether, and hot ethanol.
   2. Chloroform and sulfuric acid test for cholesterol.

Results and Discussion

The results of the two experiments are presented in table form.

EXPERIMENT I

Tissue	Grams Wet Wt.	Grams Dry Wt.	% Cephalin	% Lecithin	% Sphingomyelin	% of Total Phospholipids
S-A Node	45.1	13.5	5.63	3.85	.88	10.36
Auricle	50.0	15.0	2.93	1.73	.93	5.59
A-V Node	119.0	35.7	5.21	3.08	1.42	9.71
Ventricle	111.0	33.3	4.47	2.67	.81	7.95
Total	325.1	97.5	4.56 (Avg.)	2.83 (Avg.)	1.01 (Avg.)	8.40 (Avg.)

EXPERIMENT II

S-A Node	36.0	10.8	5.64	3.51	.64	9.79
Auricle	58.6	17.6	2.90	1.92	.73	5.55
A-V Node	122.0	36.6	5.05	3.87	.68	9.60
Ventricle	82.5	24.8	4.43	3.34	.52	8.29
Total	299.1	89.8	4.51 (Avg.)	3.16 (Avg.)	.64 (Avg.)	8.31 (Avg.)

The total percentages found lie between the 7% given by Bloor (1930, 1934), and the 9.83% given by Kaucher, et al (1943), as the amount of phospholipids in the cardiac musculature. The difference probably lies to some extent in the use of total ventricular tissue only. The results show that the sinus node has a greater per cent of phospholipids than the rest of the myocardium, with the A-V bundle a close second. There is less difference in the phospholipid content of the bundle and the ventricular tissue than there is in the sinus node and the auricle. The auricular myocardium has the least phospholipid content of the types of heart fibers. This is in accordance with Bloor's analysis of phospholipid content of tissues: "The greater the variety of activity, as well as the greater the activity, the more phospholipid content."

This does not, however, prove the greater conductivity of the bundle tissue, for there are nerve fibers in the bundle of the beef heart, and the nervous tissue is known to have a very high essential lipid content. It would be necessary to make some type of comparison of the amount of nerve fibers in th non-specialized musculature and in the bundle before a positive statement as to the activity of the muscle tissue of the bundle could be made. The same holds true for the sinus and auricular tissue. This ex-

periment does show the order of variety and the amount of activity of the areas of the heart. The sinus is most active, the bundle second, the ventricle third, and the auricular appendages last, being at least 2% behind the ventricle.

SUMMARY AND CONCLUSIONS

I. Hearts of representatives of five vertebrate classes were prepared to demonstrate nerves, connective tissue, general structure and position of the tissue assumed by some to be conductive in nature.

II. In all of the animals studied, some type of structure was found which connected either the sinus venosus or its accepted remnant, the sino-atrial node, with the ventricular musculature. The degree of the differentiation for this connecting structure varied in the different animals.

III. The connection of the pacemaker to the ventricle was most direct and most obvious in the fish, due, not to differentiation of the individual fibers, but to the arrangement of the fiber strands.

IV. There were muscular connections other than the Bundle of His from the right atrium to the right ventricle in dogs, rats, and mice.

V. A large nerve trunk, not mentioned in any previous literature, was found in the compact layer of the spade fish (Chaetodipterus faber) ventricle. Investigation of numerous species of advanced teleosts is needed to ascertain

whether this is a characteristic of all the advanced teleosts or is peculiar to the species of this study.

VI. A rich nerve supply existed in the ventricle of the turtle, alligator, and mammals. It was concluded that real distinction between afferent and efferent nerves must rely on physiological determination, since the majority of cardiac nerve fibers are non-medullated and terminal endings are so difficult to demonstrate.

VII. The nerve supply to the ventricle of mammals was extensive and diversified, including perikarya of two different types over the cranial two-thirds of the ventricular area (Davies, 1946). Perikarya were also observed in the alligator ventricle.

VIII. The conductive system of the bird appears intermediated between the reptilian and mammalian. More intensive study is needed on the bird heart before stating the course of a pathway beyond the auricular ring which connects with both auricular and ventricular myocardium.

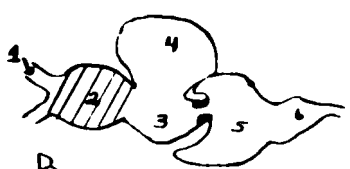
IX. More investigation correlating histological and physiological techniques is in order to determine the role of the diversified and extensive ventricular nerve supply.

X. The glycogen test is an unreliable method of tracing conductive paths or locating Purkinje fibers.

XI. A clear difference exists in the phospholipid content of the types of heart tissue.

Figure 1. Three types of fish hearts showing the electronegative sensitive centers. Type A-primitive teleosts, type B-elasmobranchs, type C-advanced teleosts. 1) Duct of Cuvier, 2) sinus venosus, 3) auricular canal, 4) auricle, 5) ventricle, 6) conus arteriosus. Sensitive centers are shaded. Data compiled from Skramlik (1930) and Bielig (1930).

A.



B.

C.

Figure 2. Photomicrograph of cells at the mitral orifice of the shark heart. A-auricular fibers, C-circular fibers at orifice, M-mitral orifice. 10% formalin, 10 micra, hematoxylin and eosin, 430X.

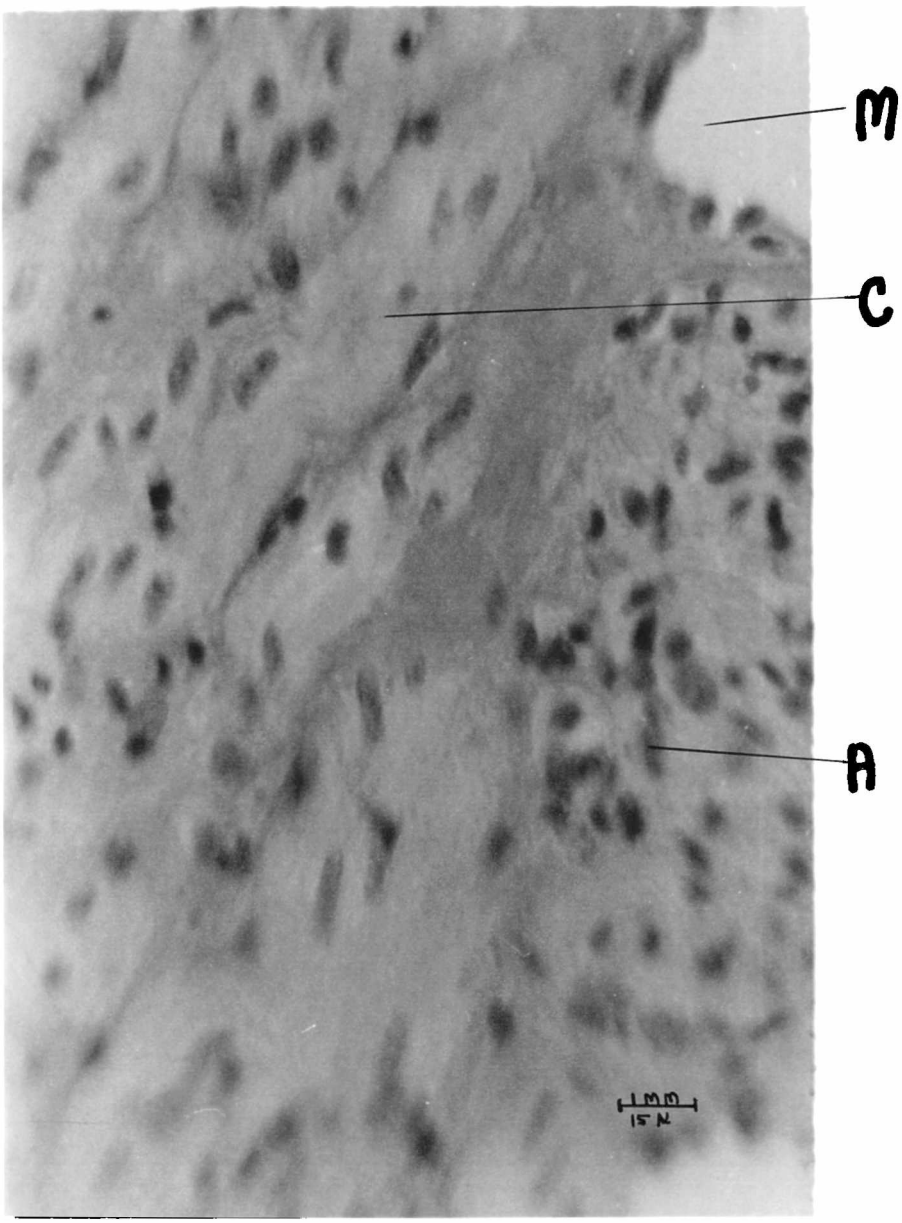

M
C
A

Figure 3. Photomicrograph of ventricle of spade fish (Chaetodipterus faber). CM-compact muscle, N-nerve trunk, SM-spongy muscle layer. 70% alcohol, 10 micra, toluidine blue, 430X.

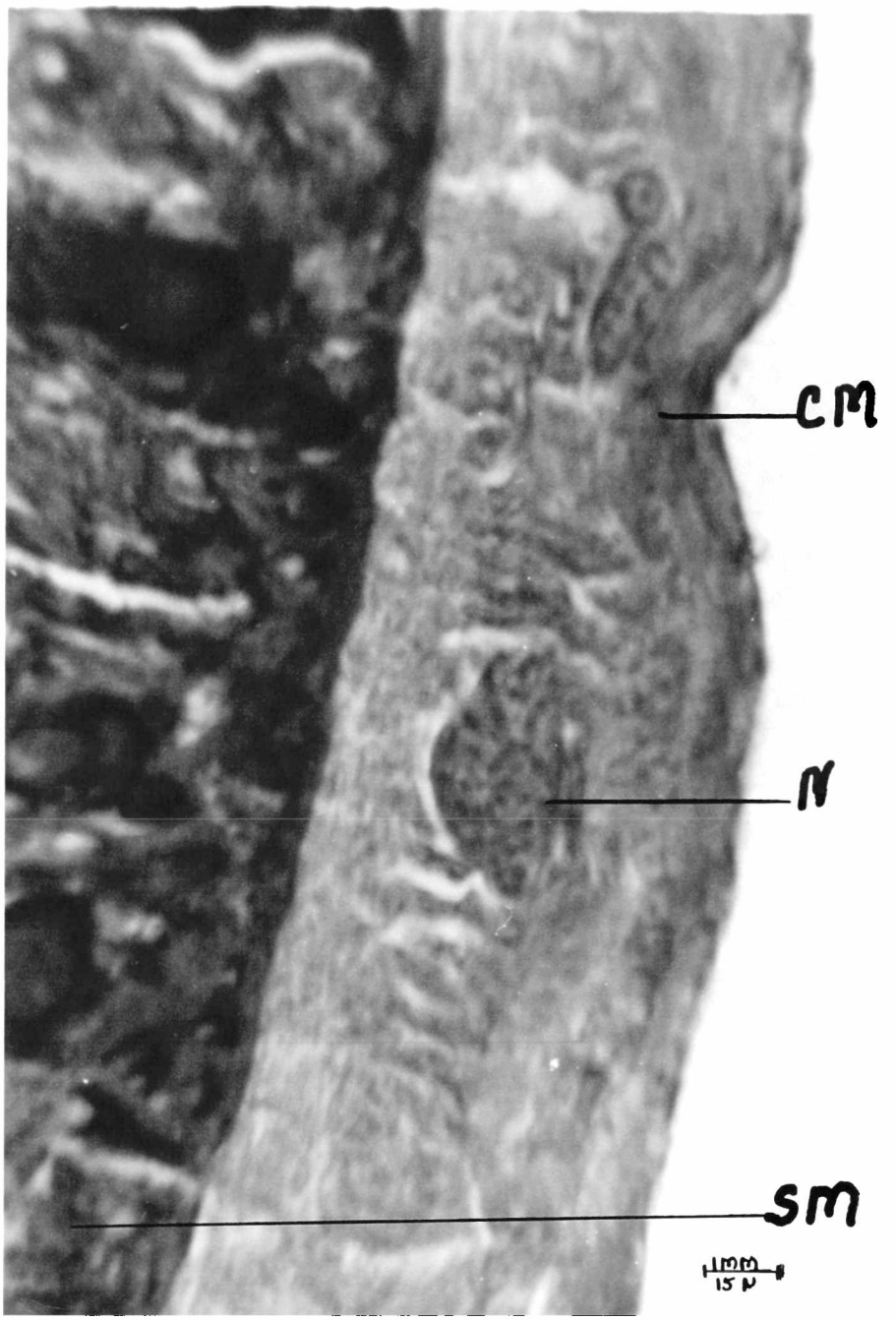

CM
N
SM
15 μ

Figure 4. Photomicrograph of frog ventricle showing reticulate appearance of musculature. B-blood, M-muscle fiber. 70% alcohol, 10 micra, toluidine blue, 100X.

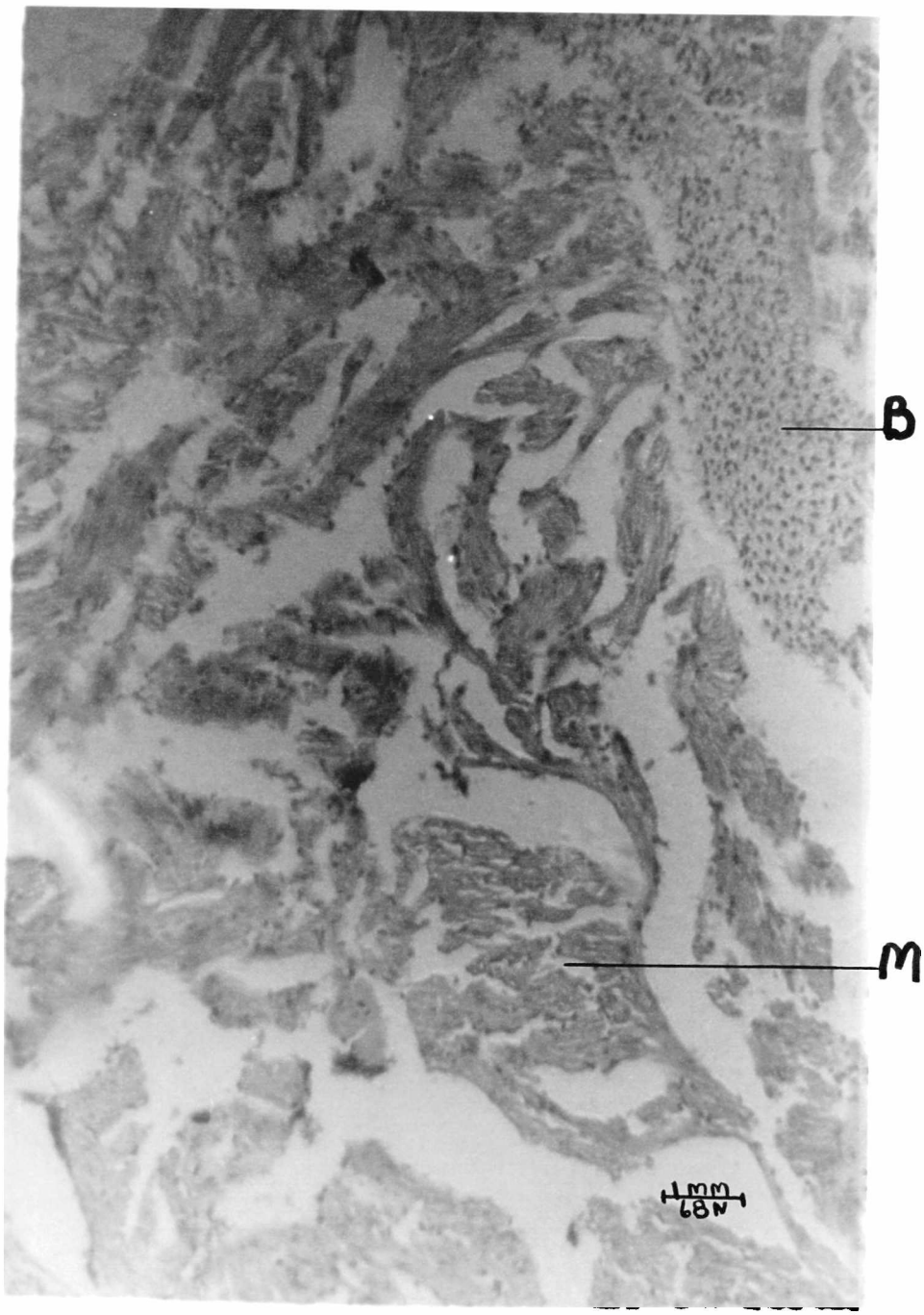

B
M
1MM
68N

Figure 5. Photomicrograph of frog ventricle showing the ventricular muscle of figure 4 magnified to 430X.

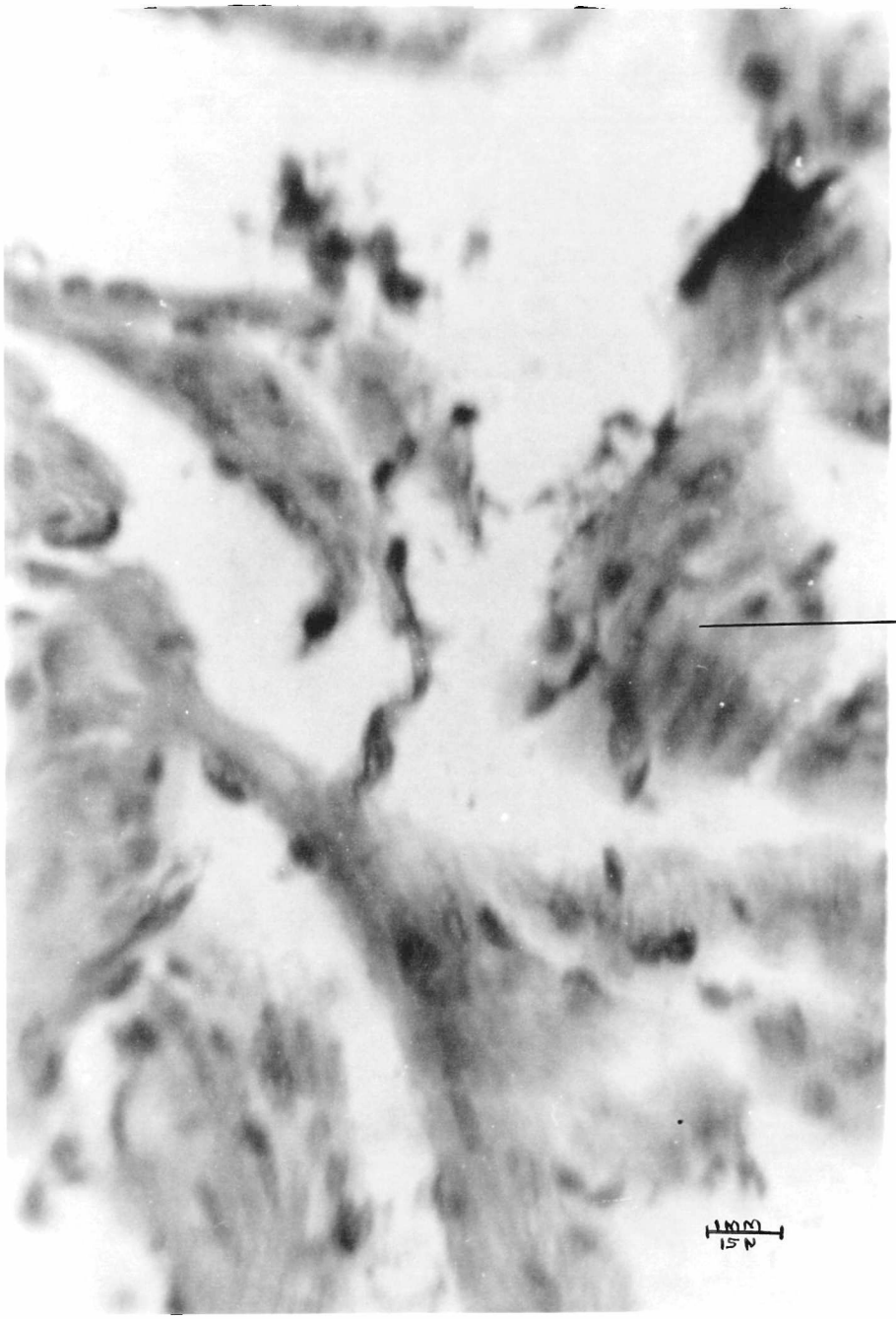

M
1MM
15 N

Figure 6. Photomicrograph of mitral orifice of turtle heart. CM--circular muscle cells at mitral orifice, DA--descending auricular cells, V--ventricular cells. 70% alcohol, 10 micra, hematoxylin and eosin, 430X.


DA
CM
V

Figure 7. Retouched photomicrograph showing the nerve fibers in the turtle ventricle. M-muscle fascicles, N-nerve fiber. 70% alcohol, 10 micra, toluidine blue, 430X.

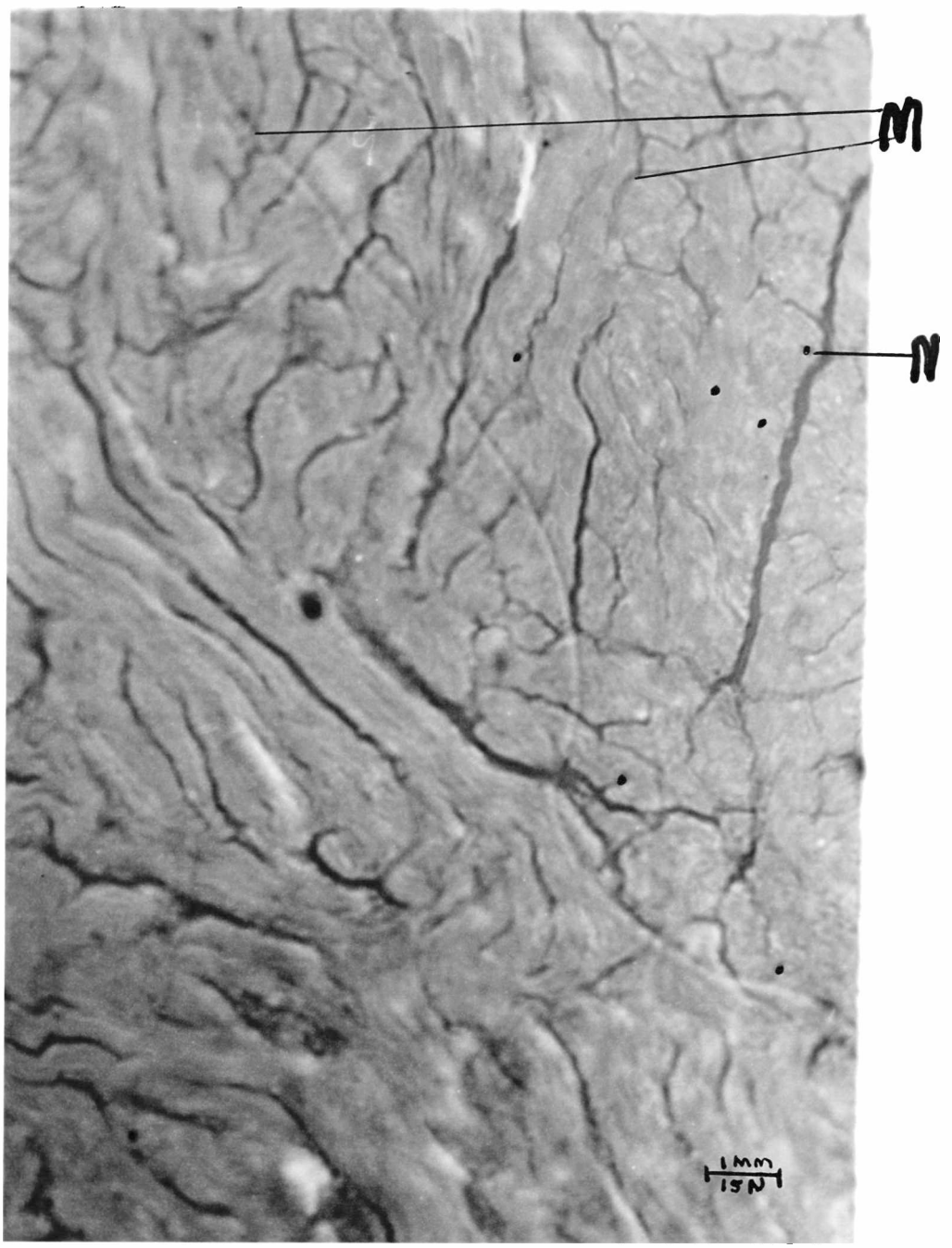

M
N
1mm
ISN

Figure 8. Photomicrograph of the alligator ventricle showing nerve fibers. M--muscle strands, N--nerves. 70% alcohol, 10 micra, toluidine blue, 430X.

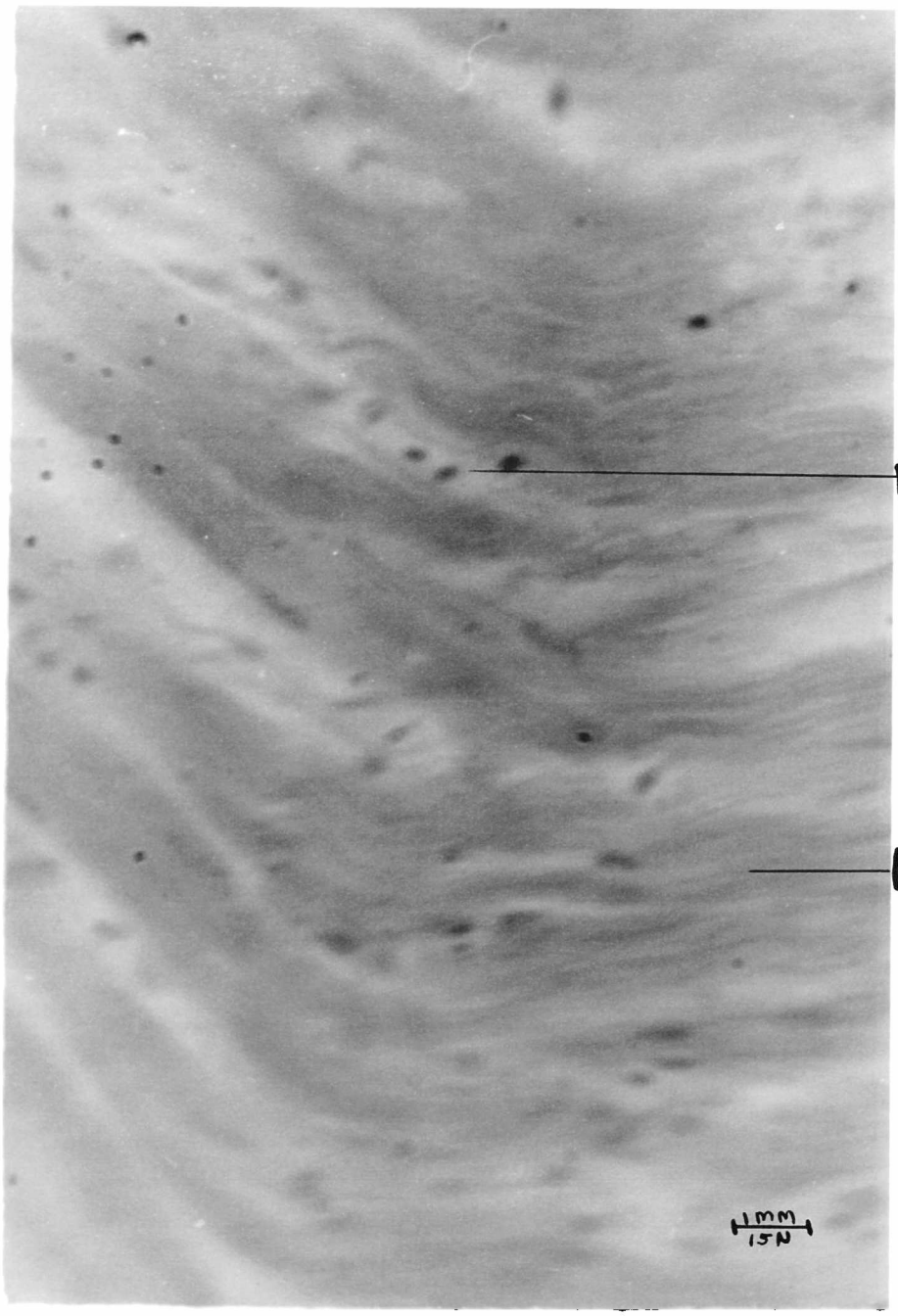

N
M

Figure 9 A. Photomicrograph of the sino-atrial node of the beef heart. AM-fibers of S-A node. 70% alcohol, 10 micra, hematoxylin and eosin, 100X.

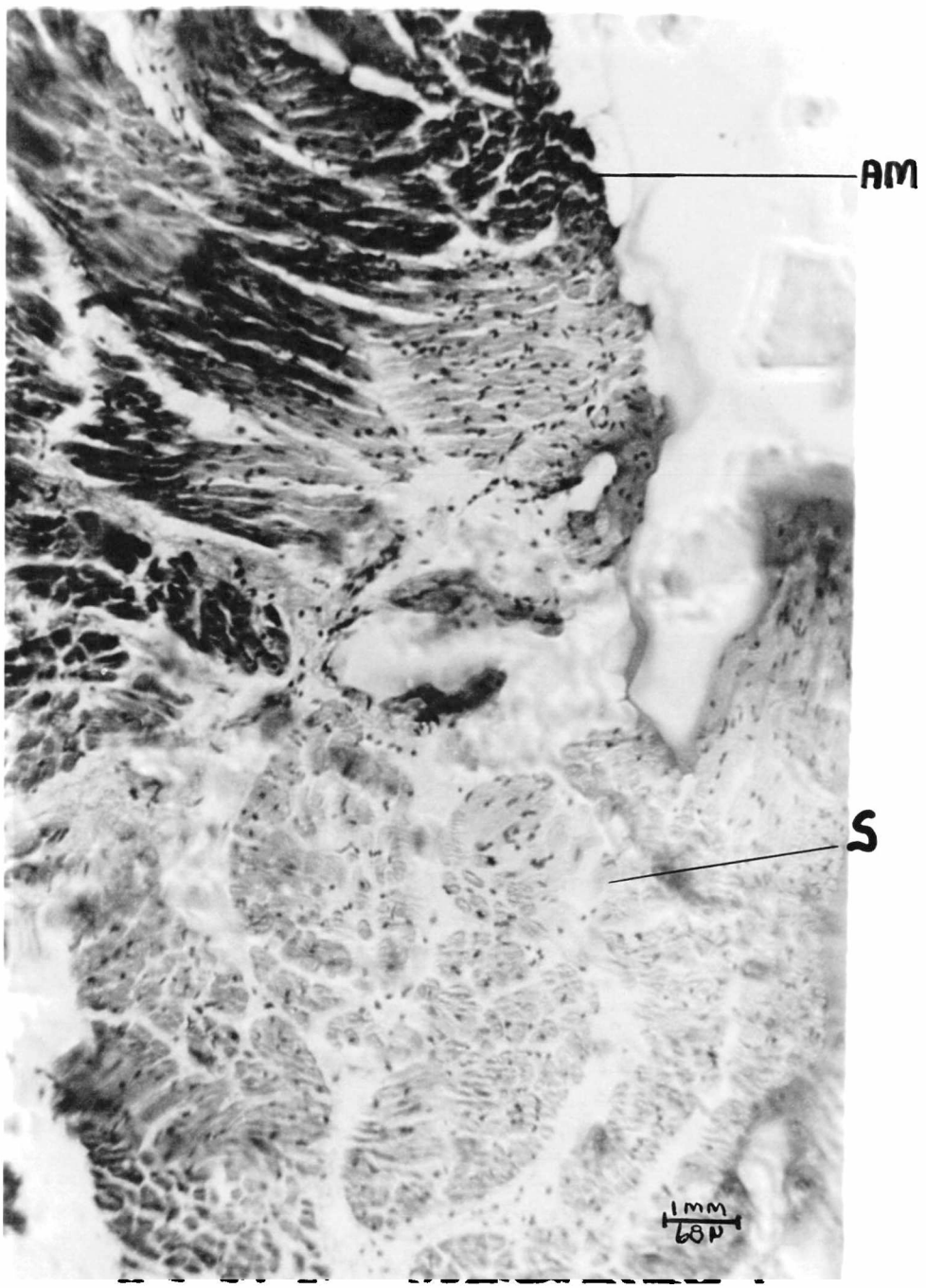

AM
S
1mm
LBP

Figure 9B. Photomicrograph of the sino-atrial node of beef heart shown in figure 9A magnified to 430X.

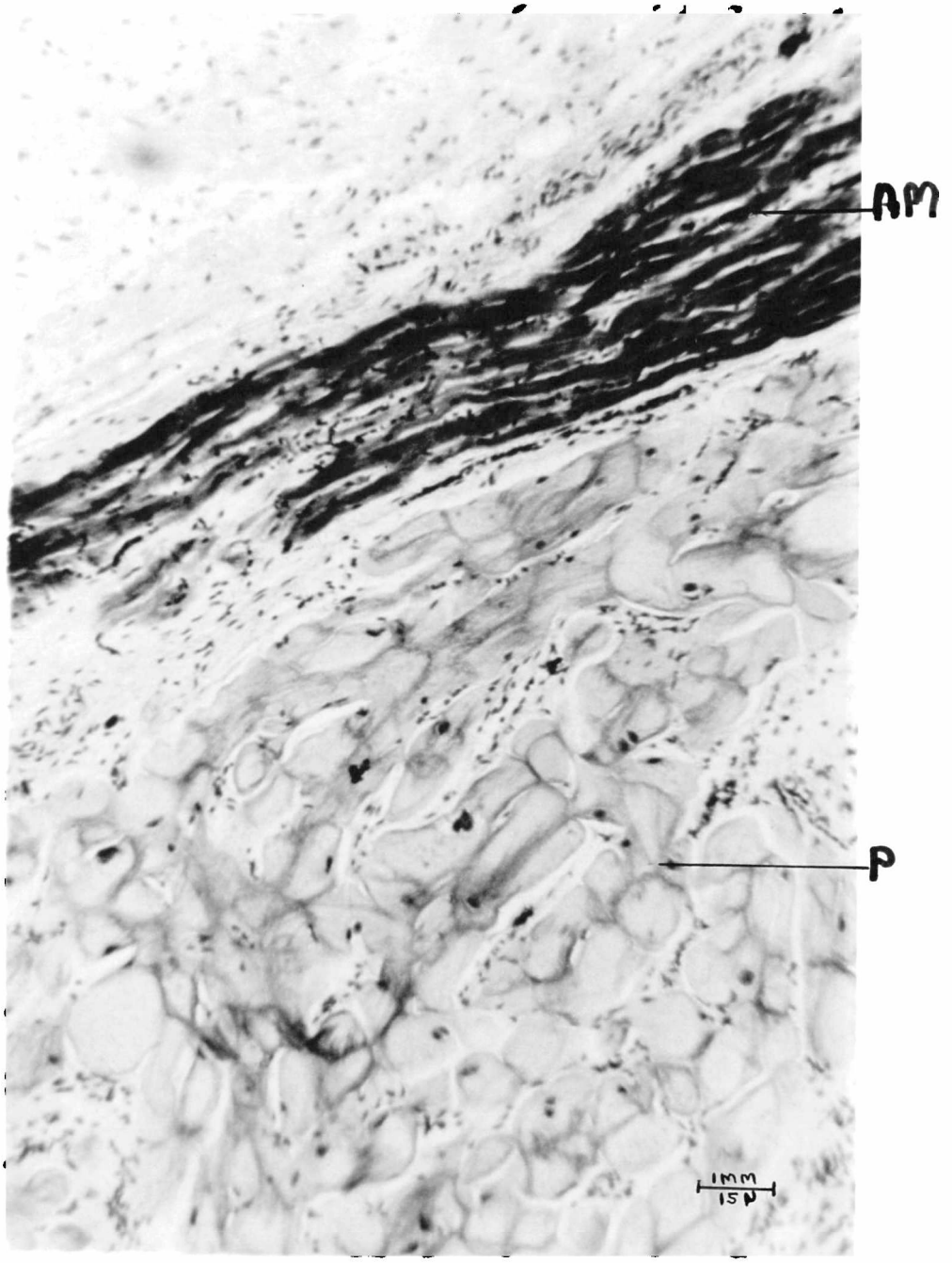

BM
P
1MM
15 μ

Figure 10. Photomicrograph of the atrioventricular bundle of the sheep heart. AM-auricular myocardium, P--Purkinje fibers. 10% formalin, 10 micra, hematoxylin and eosin, 430X.

AM
S
1MM
15 µ

Figure 11. Photomicrograph of the rat ventricle. P-Purkinje fiber, VM-ventricular myocardial cell. 10% formalin, 10 micra, hematoxylin and eosin, 430X.

P
VM

Figure 12. Camera Lucida Drawing of Purkinje cells from three different animals. A. The Purkinje fibers of the subendothelial ventricle of the rat. B. Purkinje fibers of the atrioventricular node of the sheep. C. Purkinje fibers of the sinoatrial node of the cow.

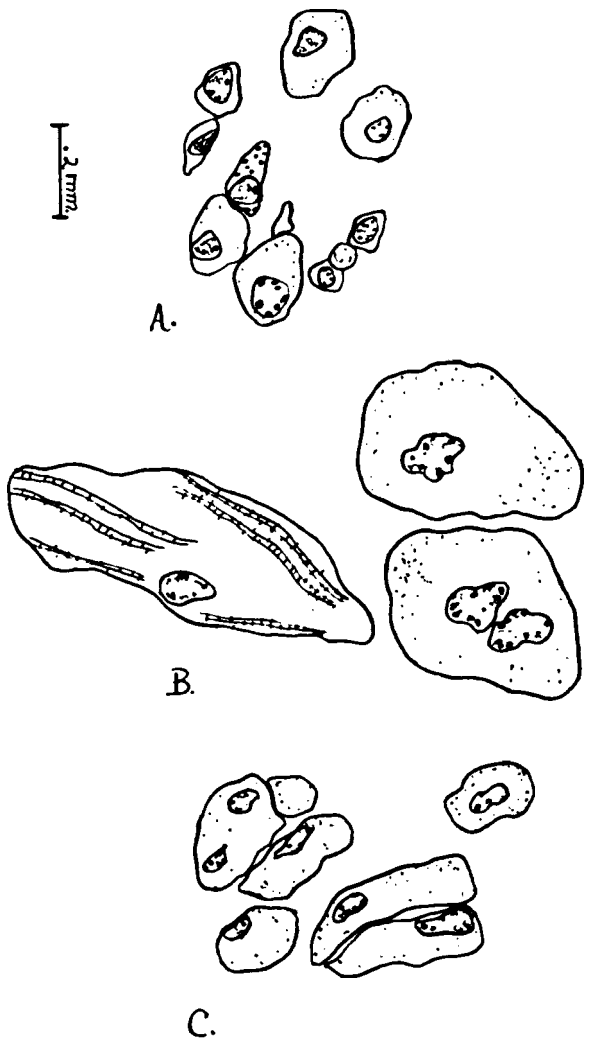
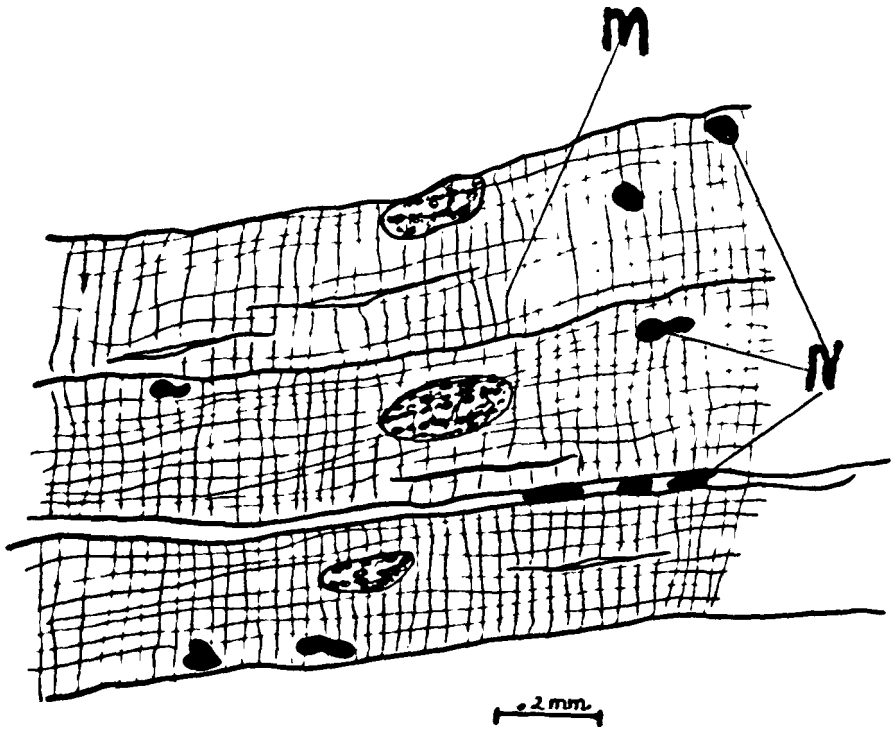

2 mm.
A.
B.
C.

Figure 13. Camera Lucida Drawing of nerves of the dog ventricle. M--muscle fiber, N--nerve fibers. 70% alcohol, 10 micra, 430X.


M
N
2 mm

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