CARDIAC INNERVATION: ANATOMIC AND PHARMACOLOGIC RELATIONS*

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ALTHOUGH it is now generally conceded that the formation and conduction of impulses in the heart occur within specialized myocardial cells, the function of these cells is profoundly influenced by the nerves of the heart. Among the many recent advances in our knowledge of cardiac electrophysiology, those obtained through electron microscopy and through intracellular microelectrode recording have notably altered old concepts. Knowing that the myocardium is composed of individual cells rather than an anatomic syncytium, for example, will have influences on electrocardiographic concepts that are only beginning to become apparent. Knowing what factors influence the action potential of membranes of single cells in specific areas of the heart inevitably helps us understand how these areas function. Ultimately we must learn which components within the cell are responsible for its electrical activity, and which components of the cell membranes participate in the formation and conduction of impulses.

Along with this essential tendency to think smaller and smaller, however, we must not forget that pacemaking in the normal heart is a far more complex operation than what can be learned from the appearance or function of a single cell. The sinus node is composed of many cells of a variety of types, including nerves, and its optimal function depends on proper synchronization of electrical discharge (simultaneously or in proper sequence) by the cells capable of spontaneous electrical activity. Furthermore, this activity must be collated with the needs of the body in terms of heart rate. Such needs are signaled by the cardiac innervation, which is the heart's principal means of rapid communication with extracardiac control centers such as the carotid body and the brain. The same considerations apply to the cells of the
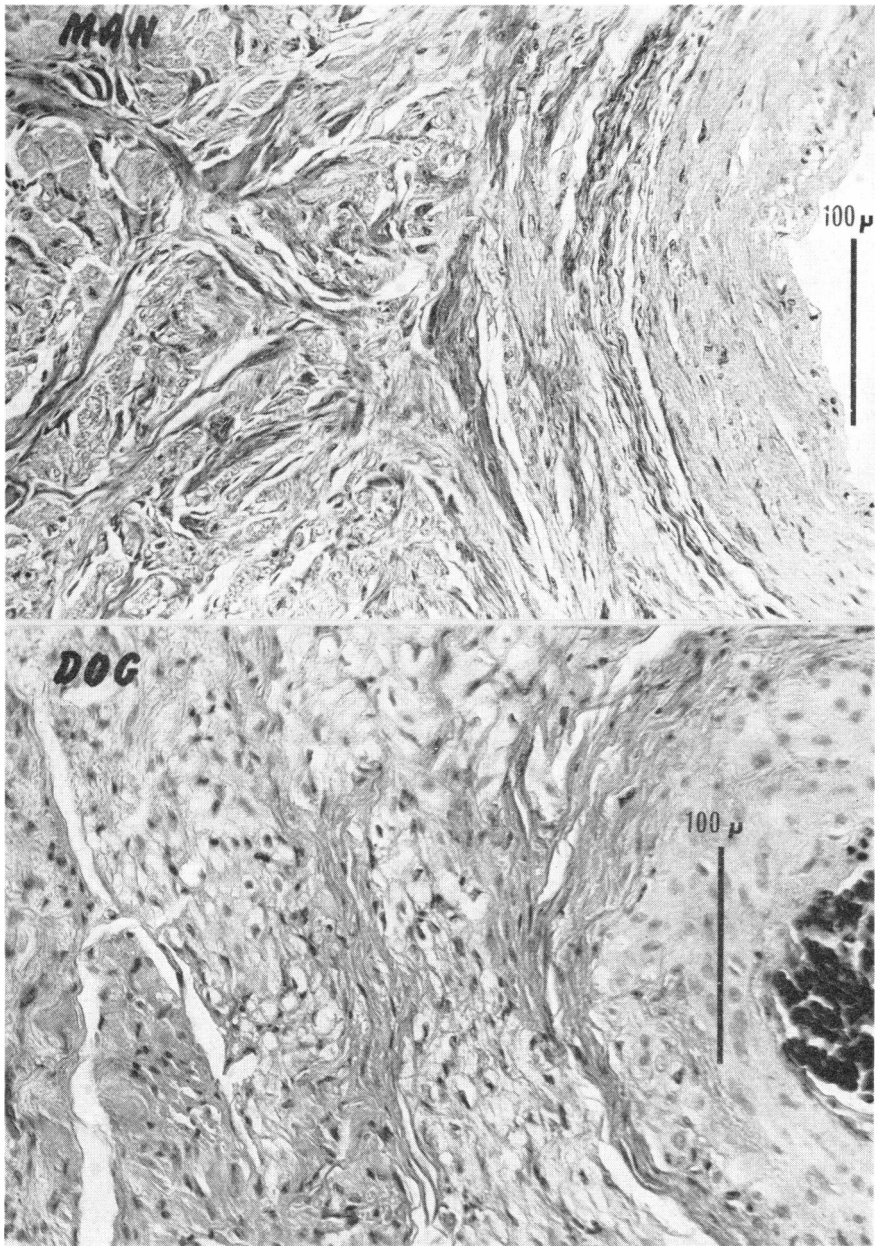
Fig. 1. These photomicrographs demonstrate the similar structure of the sinus node in man and the dog. The sinus node artery is to the right in each. This and Figures 2, 3, and 6 are from a published study[12] and are reproduced by permission of the American Heart Association.
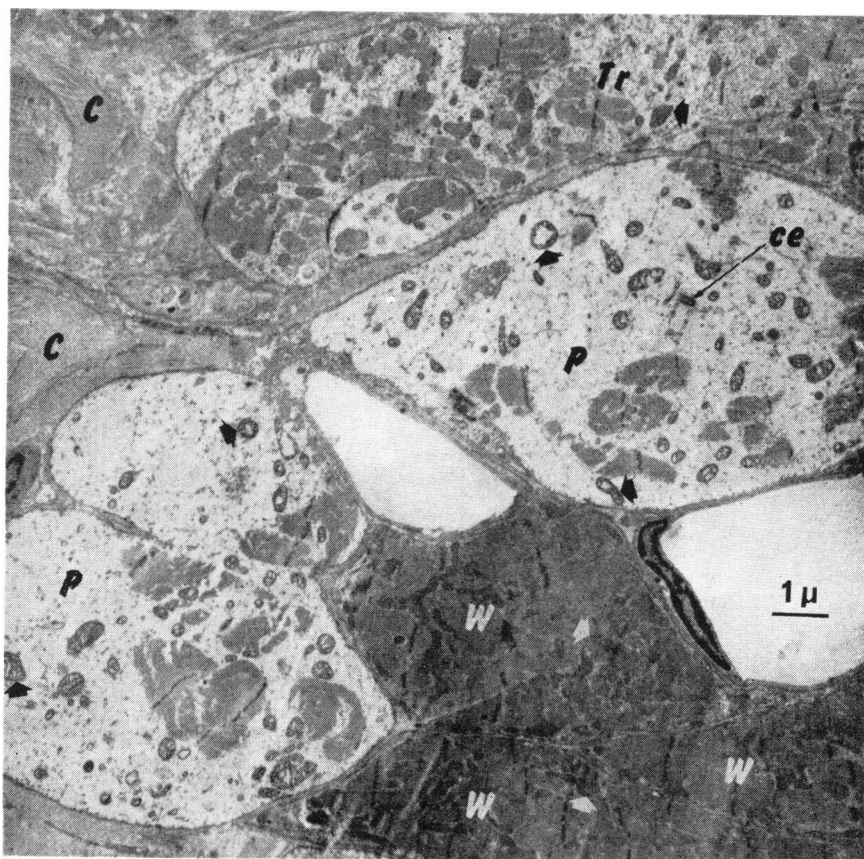
Fig. 2. This electron micrograph of canine sinus node was prepared from tissue obtained with *in vivo* fixation by direct perfusion of the node with glutaraldehyde. Characteristics of the three types of cell are discussed in the text: *P* is for P cell, *Tr* for transitional or intermediate cells, and *W* is for ordinary working myocardial cells. *C* indicates collagen and *Ce* a centriole. The small black arrows point to sarcosomes of the P cells and one in a transitional cell, while the white arrows indicate working cell sarcosomes. See also Figure 4.

atrioventricular (AV) node, the bundle of His, and Purkinje fibers.

Since the subject of this conference is arrhythmias, my presentation will deal with anatomic and pharmacologic aspects of cardiac innervation relative to the sinus node, the AV node, and the bundle of His. Other important aspects of neural control of the heart and vessels have been included in a number of recent reviews.[1-7] Gross anatomic features of cardiac innervation are familiar to most investigators and are clearly presented in standard textbooks of anatomy. For the sake of simplifying my presentation, the neural anatomy (histologic) of the cardiac con-
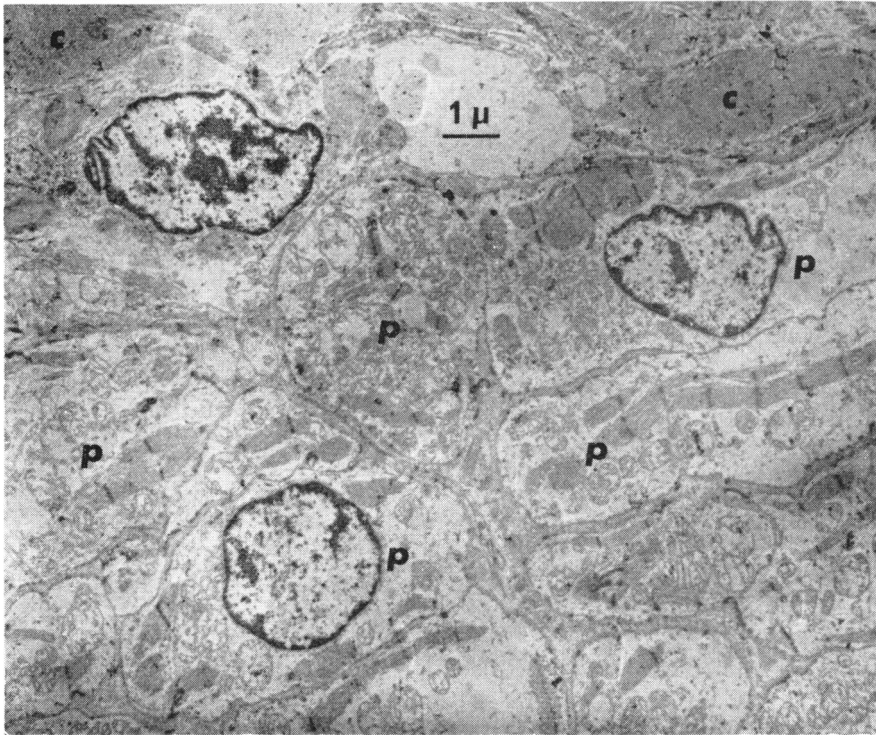
Fig. 3. This electron micrograph demonstrates that the human sinus node is similar to that of the dog at the ultrastructural level. The sarcosomes and nuclear chromatin are not as well preserved, since the specimen was obtained postmortem rather than *in vivo*, but the membranes and the intracellular organization as well as the intercellular relationships are well preserved. Abbreviations are the same as in Figure 2.

duction system will be considered first, and then its neuropharmacology. Each discussion will be governed by its pertinence to the mechanisms and management of cardiac arrhythmias.

NEURAL ANATOMY OF THE CARDIAC CONDUCTION SYSTEM

Here and in subsequent sections the principal discussion will center on observations in human and canine hearts; in the human because, for the physician, that is where the ultimate questions in biology are, and in the dog because the pharmacologic experimental studies in my laboratory have been made in this animal. The other animal used frequently by others in cardiac electrophysiologic studies is the rabbit, and comparative anatomic features will be included for the leporine heart.
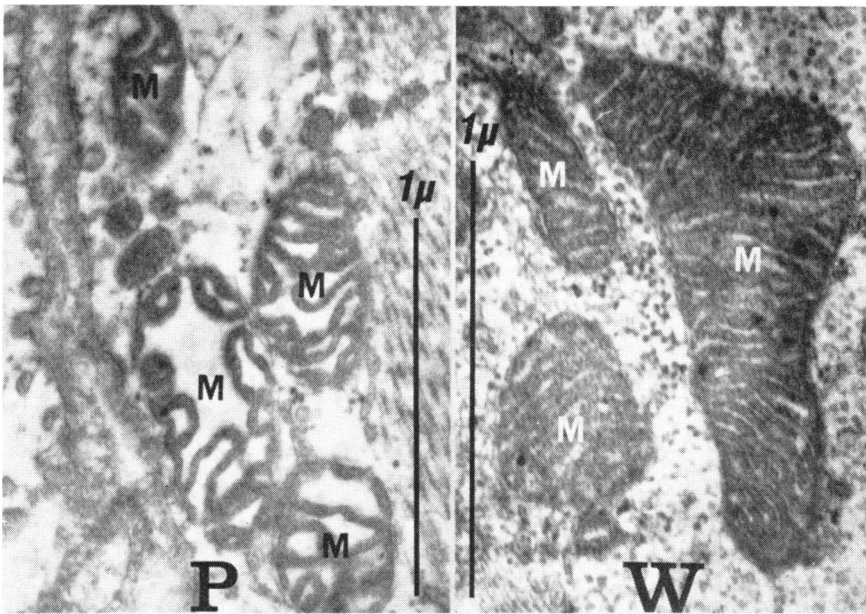
Fig. 4. This contrasts the simple structure of P cell sarcosomes (*M* on the left) with the more complex structure of working myocardial cells on the right. These specimens were obtained from the same heart at the same time in the same way, by *in vivo* glutaraldehyde perfusion of the canine sinus node.

The sinus node. The sinus node (Figure 1) is located less than a millimeter beneath the epicardium in the sulcus terminalis at the junction of the superior vena cava and right atrium. It is near the anterior margin of the atriocaval junction in man,[8] slightly more posterior than this in the dog,[9] and still further back in the rabbit,[10] where it is located approximately midway between the superior and inferior venae cavae. In man and the dog the node is organized about a conspicuously large central artery, which is not the case in either leporine or bovine hearts.[11] In human,[12] canine,[12, 13] leporine,[14, 15] and bovine[16] hearts the sinus node contains three types of cells that organize as bundles of intertwining fibers within a collagen matrix. Fibers of the sinus node under the light microscope resemble ordinary myocardium but are of smaller diameter and stain paler with all conventional dyes. Details of the individual cells are best defined with the electron microscope (Figures 2 and 3). The characteristic cell of the sinus node is the P cell,[12] which is rounded or spherical and contains relatively few myofibrils (containing few myofilaments) distributed randomly within the cell. Sarcosomes in the
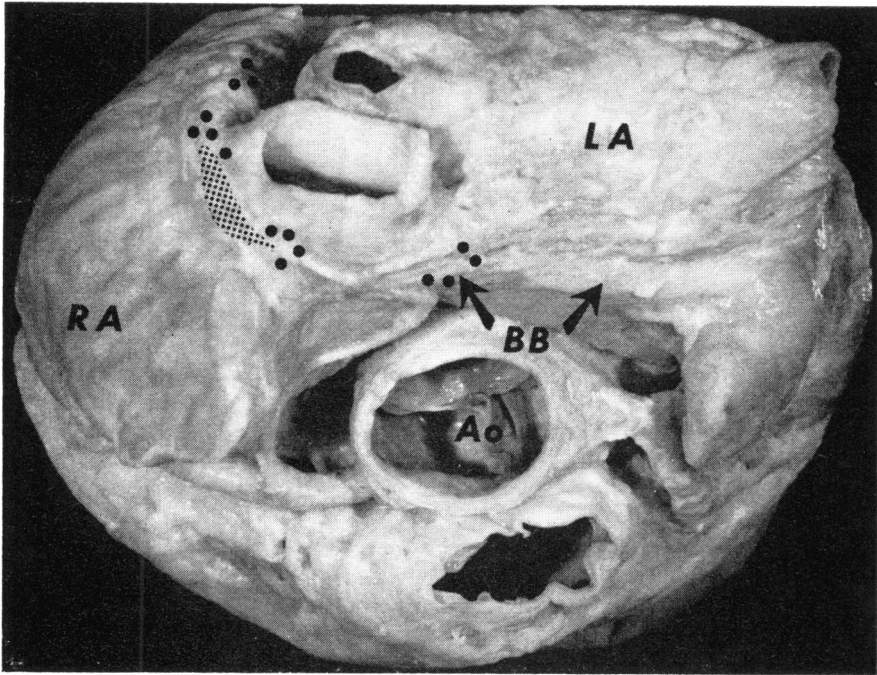
Fig. 5. In this photograph of a dissected human heart the locations of ganglia in the region of the sinus node (stippled area) are shown with dots. In addition to those at the margins of the node, two other groups are shown: those in the middle internodal tracts at the crest of the atrial septum, and those in the anterior internodal tracts below Bachmann's bundle. See also Figures 14 and 16. *LA* is left atrium; *RA* right atrium; *Ao* aorta; and *BB* with the arrows indicates Bachmann's bundle (the anterior interatrial myocardial band).

P cell are simpler than those in working myocardial cells (Figure 4) and, like the myofibrils, are distributed randomly in the P cell; there is no sandwiching arrayal between myofibrils as is characteristic of working myocardial cells. The sarcoplasmic reticulum of P cells is sparse and poorly developed. Each P cell is bound by a plasma membrane, and groups of these cells are enveloped together by a basement membrane. Between contiguous P cells there are no intercalated discs but there are scattered desmosomelike thickenings of apposing plasma membranes. No nexus formations or "tight junctions"[17] have been reported in P cells. Their cytoplasm is remarkably clear and contains surprisingly little glycogen in comparison to abundant glycogen in adjacent cells fixed the same way in the same heart at the same time. There is active pinocytosis in the plasma membranes of the P cells.

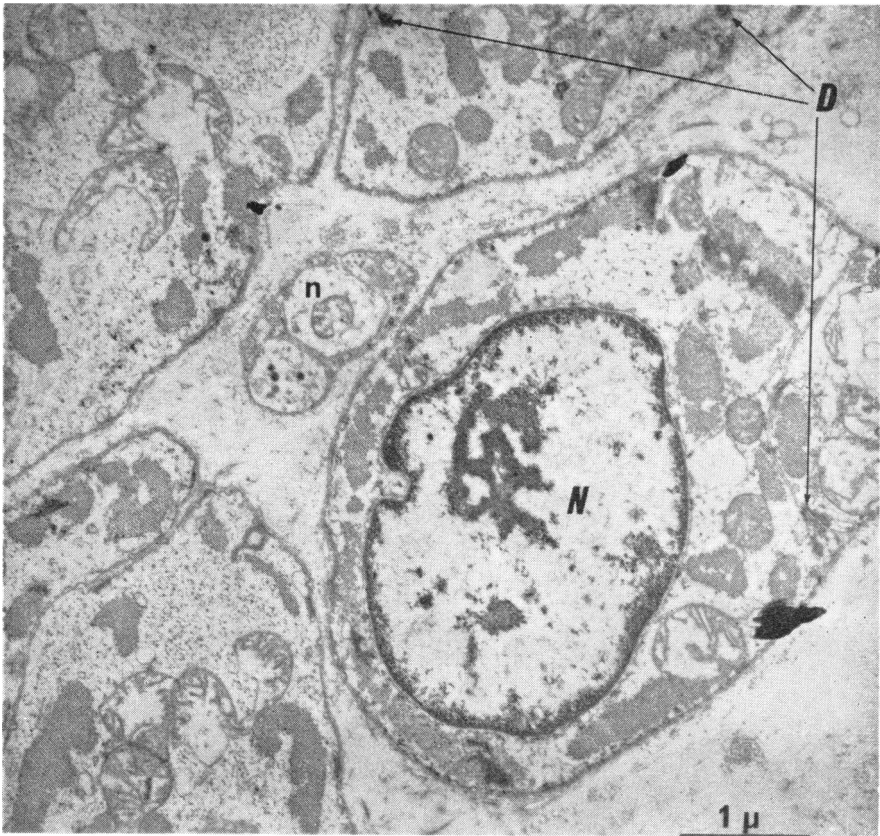The other two types of cell observed in the sinus node are ordinary

Fig. 6. In this electron micrograph the relation between a nerve terminal (*n*) and cells of the canine sinus node is shown. *D* and the arrows indicate three desmosomes. *N* overlies a nucleus. Active pinocytosis is visible in the cell membranes.

working myocardial cells, which are in highest concentration near the margins of the node but occur even in some central locations, and a transitional cell that has internal structure intermediate between that of the P cell and the working cell. The transitional cell also serves as the only connection between the other two types of cell; no P cells are observed connected directly to working myocardial cells.

Nerves and ganglia are abundant in the region of the sinus node (Figure 5). With very rare exceptions there are no ganglion cells within the node; these cells are distributed almost exclusively at its anterior and posterior margins in the sulcus terminalis or along its caval (not atrial) margin. The nerves within the sinus node, as elsewhere in the myocardium, do *not* terminate directly on the cell membrane but
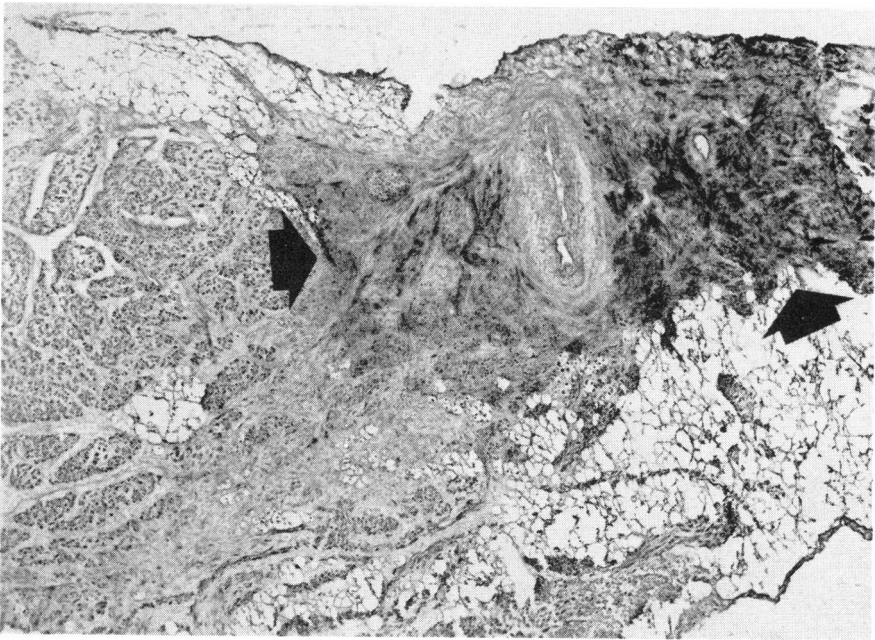
Fig. 7. Cholinesterase in the human sinus node (between arrows) stains in heavy contrast to that in neighboring atrial myocardium. Note absence of cholinesterase in the smooth muscle of the sinus node artery. Epicardium is above and the superior vena cava is to the right (×30).

at some distance (electron microscopically) from it (Figure 6). This is a significant difference from the situation in skeletal muscle, where nerve endings are not only directly on the membrane but have a special structure. Both the observations on nerve endings and on the question of nexus formation must be qualified, however, by the possibility that further studies may in time reveal different findings. What can be said on the basis of present evidence is that such structures, if they exist, are rare. Functionally this point is of considerable importance, since one must conclude on the basis of present observations that the effects of neurotransmitter substances begin after diffusion across a finite space to reach the myocardial cell surface.

Indirect evidence of the cholinergic innervation of the sinus node may be obtained with stains for cholinesterase.[18, 19] In the human heart cholinesterase is abundant within the sinus node, which stains as a dark island sharply contrasting with its surrounding atrial myocardium (Figure 7). Another striking contrast is the absence of cholinesterase

Fig. 8. The distribution of cholinesterase within the cells of the human sinus node is shown here. That associated with myofibrils is most easily identified at this magnification. In *A* (×175) the difference in staining depth of adrenergic nerves (*a*) and cholinergic nerves (*c*) is apparent, the latter being much darker. In *B* (×450) the open arrows indicate P cells, with the randomly distributed myofibrils clearly shown, and the black arrow indicates a cholinergic nerve.

in the smooth muscle of the centrally located sinus node artery. Cholin-
esterase is distributed within all three types of cell in the sinus node
and there is no detectable difference in cellular concentration. Intra-
cellular cholinesterase is readily identifiable in the randomly distributed
myofibrils of P cells as well as the parallel myofibrils of other cells
(Figure 8). It also occurs in a number of other intracellular organelles;
more specific identification of it was not possible with the light micro-
scope. Nerve endings within the sinus node stained either very deeply
(much more than the myocardial cells), or approximately to the same
intensity. Koelle[20] suggests that such difference in the depth of cholin-
esterase staining may be a useful means for anatomic differentiation of
adrenergic (light staining) and cholinergic (deep staining) nerve ter-
minals. Since there is such a large number of different enzymes con-
cerned with norepinephrine degradation *in vivo*, it may not be possible
to utilize histochemical methods to identify adrenergic nerve endings
as reliably as cholinergic ones, although recent observations by Muller
and Pearse[21] give some promise in this direction.

The AV node and the bundle of His. In man[22] and the dog[23] the
AV node is located less than a millimeter beneath the right atrial endo-
cardium just anterior to the coronary sinus ostium and just above the
insertion of the septal leaflet of the tricuspid valve. In the rabbit the
AV node is considerably displaced anteriorly by an especially large
coronary sinus present because the rabbit normally has a left superior
vena cava.[10] If one accepts Patten's theory[24] on embryologic origin of
the AV node (that it began its existence at the junction of the primitive
left anterior cardinal vein with the sinus venosus and later migrated
into the junction of interatrial and interventricular septa with the dorsal
endocardial cushion as the sinus venosus is absorbed into the adult
atria), then persistence of the left superior vena cava in the rabbit
instead of its attrition into an oblique vein of Marshall, as in man and
the dog, may indicate a more primitive condition for the AV node
in the rabbit.

In contrast to the sinus node, the AV node is not organized about
a central artery, although one is sometimes seen within it. There is
much less collagen within the AV node than the sinus node. The cells
of the AV node are composed into interweaving fibers that mingle both
singly and in bundles (Figure 9). The fibers are slightly thicker than
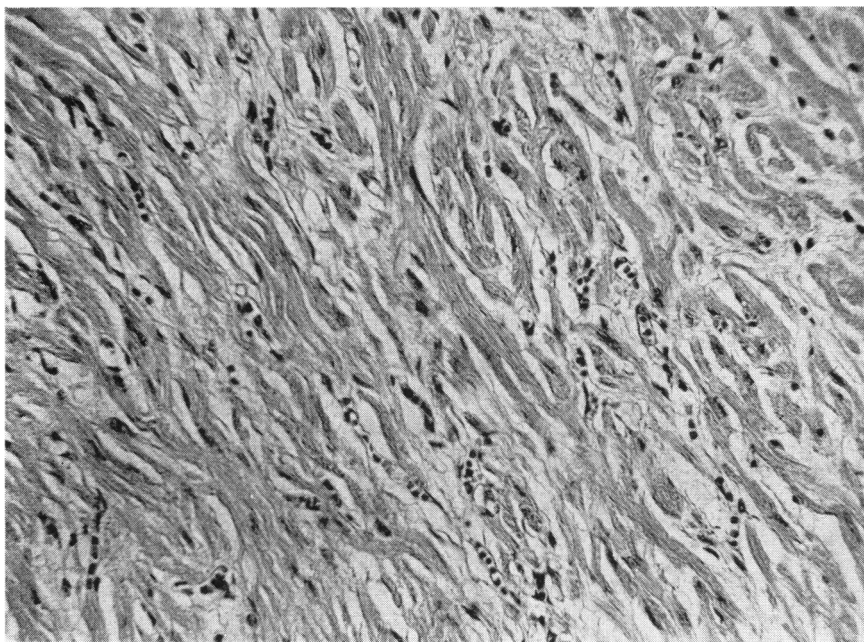those of the sinus node. The AV node has a convex surface directed

Fig. 9. This photomicrograph demonstrates the cellular structure of the human AV node ($\times$325). Compare with Figure 1, which shows the sinus node, and Figures 12, 13, and 15, which show other aspects of the AV node.

toward the right atrium and a concave surface that rests on the mitral annulus or central fibrous body. At its anterior inferior margin the fibers of the AV node begin to interweave less and orient more longitudinally as they converge to form the bundle of His, which veers centrally into the central fibrous body and then down along the posterior and inferior margin of the membranous interventricular septum. The longitudinally oriented fibers within the bundle vary a great deal in diameter, some being as large as typical Purkinje cells and others as small as fibers of the AV node. Such variation is principally in different hearts, since in a given heart the fibers of the bundle of His tend to be of uniform diameter. In either case these fibers course in groups about which there is a dense collagen sheath; they compartment the bundle of His into many separate smaller bundles (Figure 10). There is furthermore a delicate single septation about many of the individual fibers within these smaller bundles (Figure 11). The possible electrophysiologic significance of such anatomic compartmentalization has been recently reviewed relatively to several old questions in clinical electro-
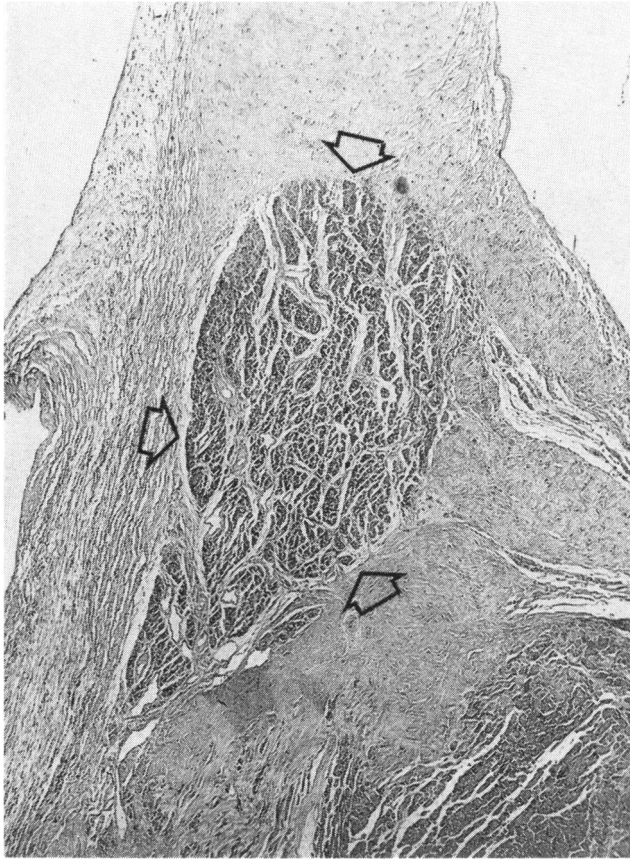
Fig. 10. This low power ($\times 45$) photomicrograph shows the collagenous partitioning of the normal human AV (His) bundle, which is bracketed by the three arrows. The tricuspid valve is visible at the left, the central fibrous body above and the inter-ventricular septum below. This and Figure 11 are reproduced from the *Henry Ford Hospital Medical Bulletin*[25] with permission.

cardiography,[25] including the genesis of the ventricular preexcitation (Wolff-Parkinson-White) syndrome.

Shortly after the bundle of His emerges from the inferior margin of the central fibrous body to reach the crest of the ventricular septum, it divides into right and left bundle branches. There is considerable variation in the relative position of the slender right branch of the bundle in the course of this division, but the left bundle system is always a wide sheet of fibers that receives most of the substance of the undivided common bundle. One may accurately think of the bundle of His and its left branch of the bundle as a single continuum from
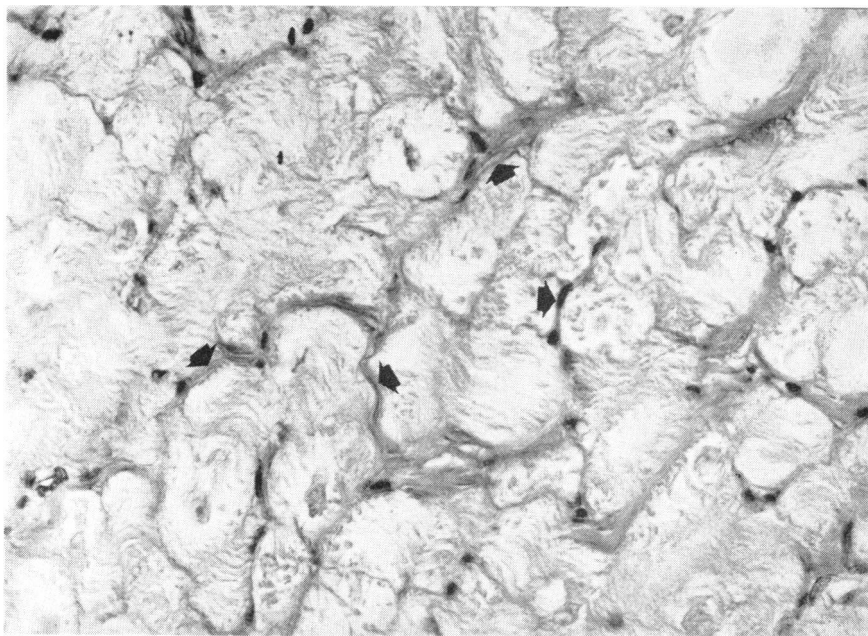
Fig. 11. This is a section of canine bundle of His ($\times$1275) demonstrating fine collagen septa, some indicated by short black arrows, separating single and small groups of fibers. The same arrangement exists in the human bundle of His.

which the right branch of the bundle is a small and variably placed division.

From preliminary examination of the human AV node and its neighboring structures with the electron microscope, and based on similar examinations by others in the dog,[13] rabbit,[14] and cow,[16] it bears many similarities to and some differences from the sinus node. There are P cells in the AV node but not in as great concentration as in the sinus node (Figure 12). Their internal structure and relation to transitional and working myocardial cells are similar to those in the sinus node. Cells in the bundle of His and the branches of it that appear under the light microscope as a large perinuclear clear zone of "Purkinje fibers" resemble rather large P cells, and they may function in the same way as those in the sinus node, viz., as pacemaking sites. Whether the sarcosomes in these cells have the unique appearance characteristic of those in the sinus node is not yet certain.

Just as in the sinus node, the AV node does not normally contain ganglia but is richly innervated. The ganglia near the AV node are
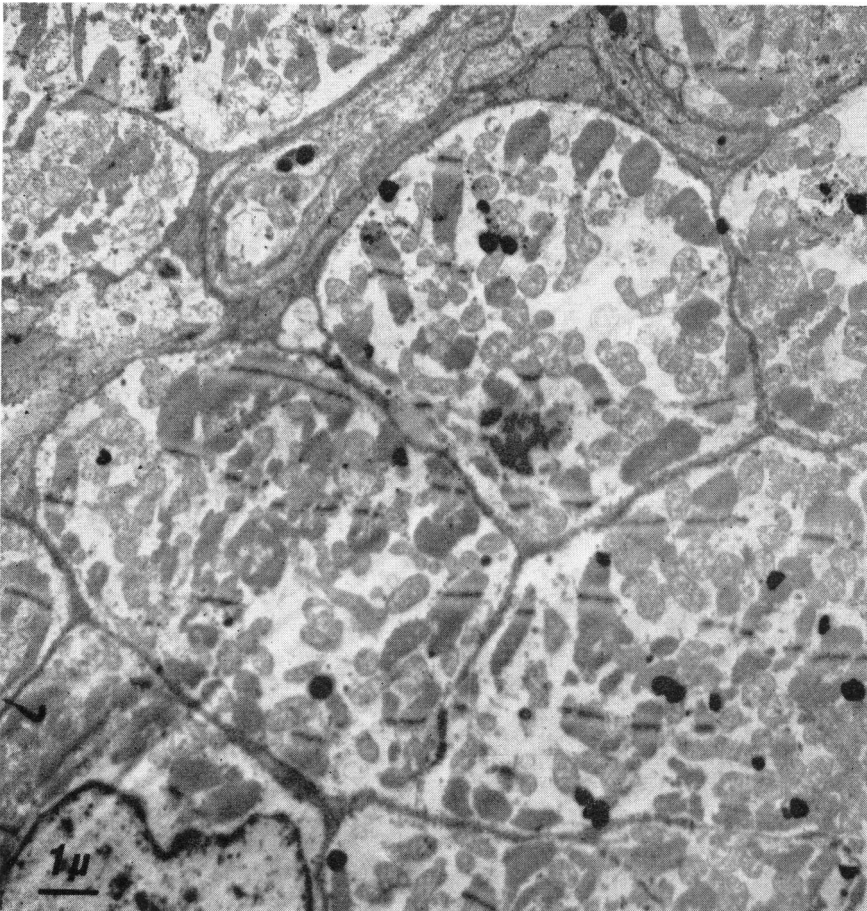
Fig. 12. This electron micrograph of human AV node demonstrates a cluster of P cells similar to those present in the sinus node (Figure 3). The densest small black bodies are artefacts.

concentrated at its posterior margin, lying between it and the anterior wall of the coronary sinus.[22, 23] It is of considerable physiologic significance that this is the exact region demonstrated by Juhasz-Nagy and Szentivanyi[26] to be of such importance in the von Bezold-Jarisch reflex. Nerves in the AV node or the bundle of His are so fine in man and the dog that they are difficult to identify without special staining; in the rabbit, on the other hand, relatively large and easily identified nerves course through the AV node and the bundle of His.

Cholinesterase (Figure 13) is heavily concentrated within the AV node and the bundle of His[18, 19] as it is in the sinus node. The intra-
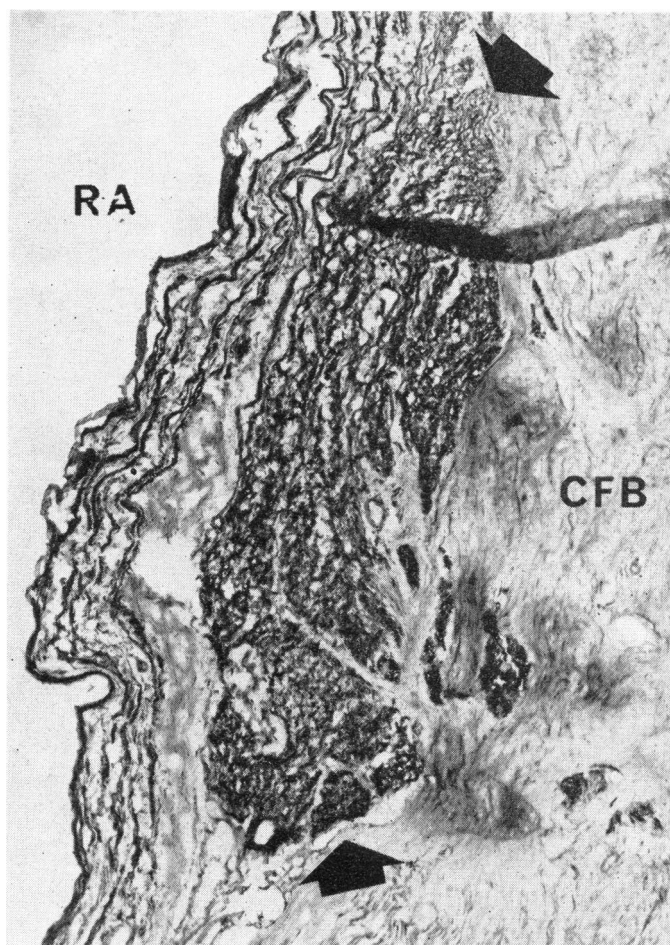
Fig. 13. The human AV node (between the two arrows) contains abundant cholinesterase. Absence of cholinesterase in the central fibrous body at the right margin of the photograph is in sharp contrast. *RA* is cavity of right atrium and *CFB* is central terae. Absence of cholinesterase in the central fibrous body at the right margin of the contain abundant cholinesterase. Magnification is ×45. This figure is reproduced from the *Anatomical Record*[19] with permission of the Wistar Institute.

cellular distribution and the relative concentration in different nerve endings is also comparable to that observed in the sinus node. At the ultrastructural level we have not observed nerve endings to terminate directly on the membranes of cells in the AV node or the bundle of His. Cholinesterase is present in high concentration in the proximal bundle branches.

The internodal pathways. In the hearts of man,[27] the dog,[23] the
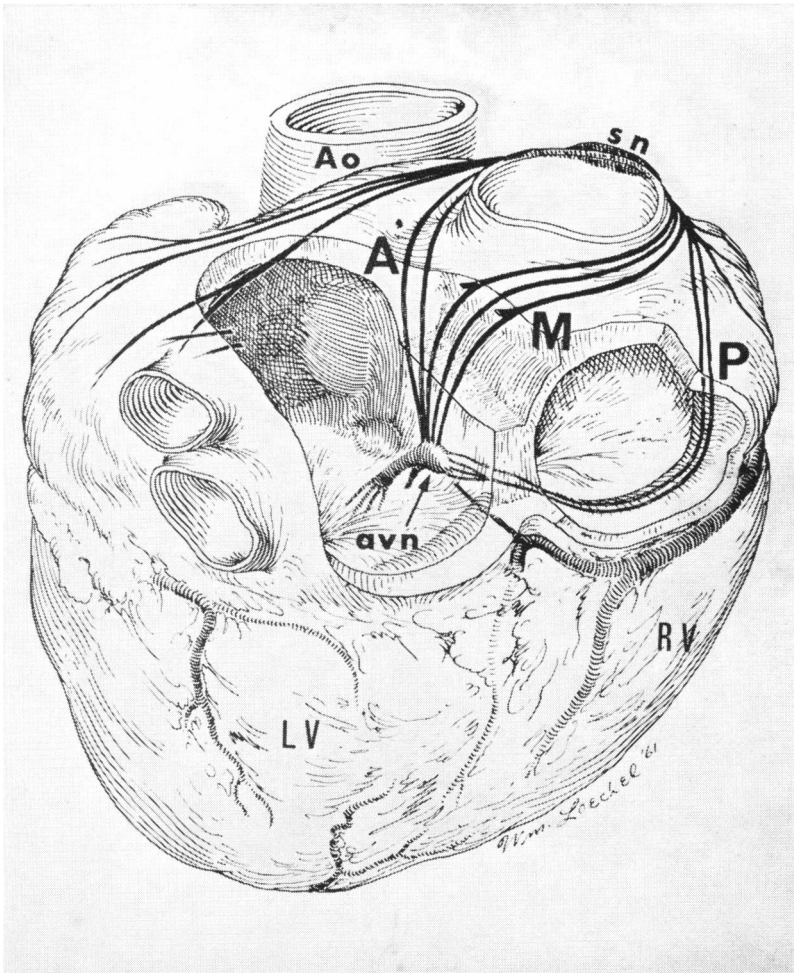
Fig. 14. This drawing illustrates the three internodal pathways described in the text. The heart is viewed from above and behind the left atrium. The abbreviations are as follows: *A*, anterior internodal tract; *M*, middle internodal tract; *P*, posterior internodal tract; *LV*, left ventricle; *RV*, right ventricle; *Ao*, aorta; *sn*, sinus node; *avn*, AV node.

steer,[11] and the rabbit[10] there are distinct bundles of continuous fibers that connect the sinus node and AV node, and that have been demonstrated physiologically to conduct more rapidly than ordinary myocardium.[28-33] There are large numbers of cells with Purkinje characteristics within these pathways (or tracts), but they are not composed exclusively of such cells. The internodal pathways exist in three divisions: the anterior, middle, and posterior (Figure 14). The *anterior*

Fig. 15. *A* is a photomicrograph (×17) of human AV node demonstrating its relation to the mitral annulus and central fibrous body (*M*), and depicting the input system to the node. *T* labels the tricuspid value. The atrial septum is above, and fibers descending in the middle (principally from anterior and middle internodal tracts) decussate with those coursing under the right atrial endocardium (principally from the posterior internodal tract). Some of these enter the superior margin of the AV node while others course along its convex surface to its inferior margin; these junctions were confirmed with serial sections. In *B* (×300) one can see the contrasting appearance between fibers in the bypass tract (most of the right half) and in the AV node (left half). Those in the bypass area have characteristic features of Purkinje cells. Part *A* of this figure is reproduced from the *American Heart Jnl.*[7] with permission of C. V. Mosby Co.

internodal pathway is similar in all four species and includes the interatrial pathway first described by Bachmann[34] but, in addition, a portion within the atrial septum that Bachmann did not describe. Fibers in this pathway leave the anterior margin of the sinus node and course forward about the superior vena cava to enter the anterior interatrial myocardial band; there they divide into two groups, those coursing on into the left atrium (Bachmann's bundle) and those curving back into the interatrial septum and descending to the crest of the AV node. The *middle internodal pathway* is also anatomically similar in all four species, coursing from the posterior margin of the sinus node behind the superior vena cava and across the sinus intercavarum to descend along the right atrial side of the septum into the crest of the AV node. The middle internodal tract corresponds to that originally described

by Wenckebach,[35,36] except that the portion crossing to the left atrium over the septum to connect the two atria is rarely as well developed as he indicated. The *posterior internodal pathway* also leaves the posterior margin of the sinus node but then courses along the crista terminalis and through the Eustachian ridge into the posterior margin of the AV node principally by crossing over the coronary sinus in man. However, in all species some of these fibers may course from the crista terminalis under the inferior vena caval orifice and under the ostium of the coronary sinus to reach the posterior margin of the AV node. In the rabbit, where the ostium of the coronary sinus is so large, the portion going under it may be the main one, corresponding to that described by Paes de Carvalho et al.[37] The posterior internodal pathway corresponds in general to that originally described by Thorel[38, 39] and in most species is anatomically the longest of the three internodal routes.

In the region of the AV node, fibers from all three pathways converge on the node superiorly and posteriorly; however, some of these fibers do not enter the nodal crest but descend along the convex margin to enter the more anterior and inferior portion of the node near its junction with the bundle of His (Figure 15). If one assumes that the point of maximal delay in AV transmission is at the superior atrionodal junction (the crest), then the fibers coursing directly under the right atrial endocardium to enter the convex margin of the AV node may physiologically as well as anatomically *bypass* the point of delay. For anatomic reasons these fibers have been indicated as bypass tracts,[22] and although their physiologic significance remains to be established, they may be important anatomic components of such physiologic phenomena as dual routes of AV transmissions[40] and of such clinical electrocardiographic events as reciprocal rhythm and ventricular preexcitation.[25]

From the description of the location of ganglia relative to the sinus node and AV node it is apparent that these lie directly within or adjacent to the various internodal pathways. In addition to those ganglia near the anterior and posterior margins of the sinus node, and at the posterior margin of the AV node, there is frequently a large concentration of ganglia within the middle internodal pathway near the crest of the interatrial septum, and another group within the anterior internodal pathway in its course just behind the aorta (Figures 5 and 16). The concentration of cholinesterase within these pathways is not remarkably different from that in ordinary atrial myocardium, except in
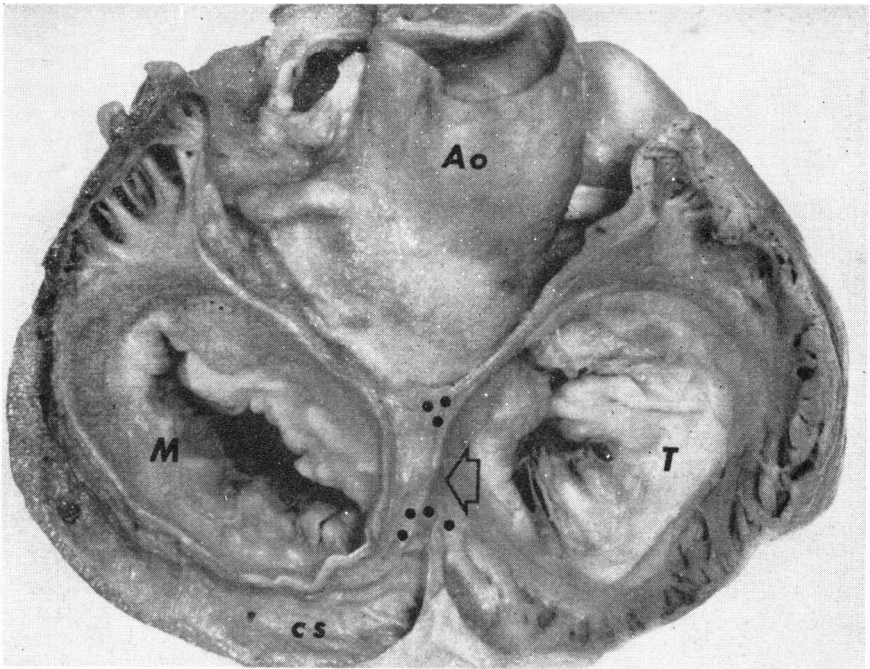
Fig. 16. In this photograph of dissected normal human heart the upper portions of both atria and atrial septum have been removed, and the coronary sinus exposed. The black dot clusters indicate the location of ganglia, with the upper group placed in the anterior internodal tract and the lower group within the posterior internodal tract just behind the AV node (open arrow). Compare with Figures 5 and 14. *M* is mitral valve; *T* tricuspid valve; *Ao* aorta; and *cs* coronary sinus.

the cells of the bypass tract (Figures 13 and 15). The possible functional importance of vagal control of conduction within the bypass tract is supported by the presence of more stainable cholinesterase there.

Both in the internodal pathways and in atrial myocardium generally there is focal variation in the concentration of cholinesterase. Some groups of cells contain more cholinesterase than neighboring groups. This inhomogeneity of cholinesterase staining sometimes but not always corresponds to identifiable cholinergic nerve endings. The anatomic variability of cholinesterase distribution supports the physiologic evidence[41] of inhomogeneity in vagal influence on the atria, a point of considerable importance in understanding the genesis and maintenance of certain arrhythmias such as atrial fibrillation.[42]

Neuropathology of the cardiac conduction system. Some diseases associated with clinically apparent extracardiac neural disease are also
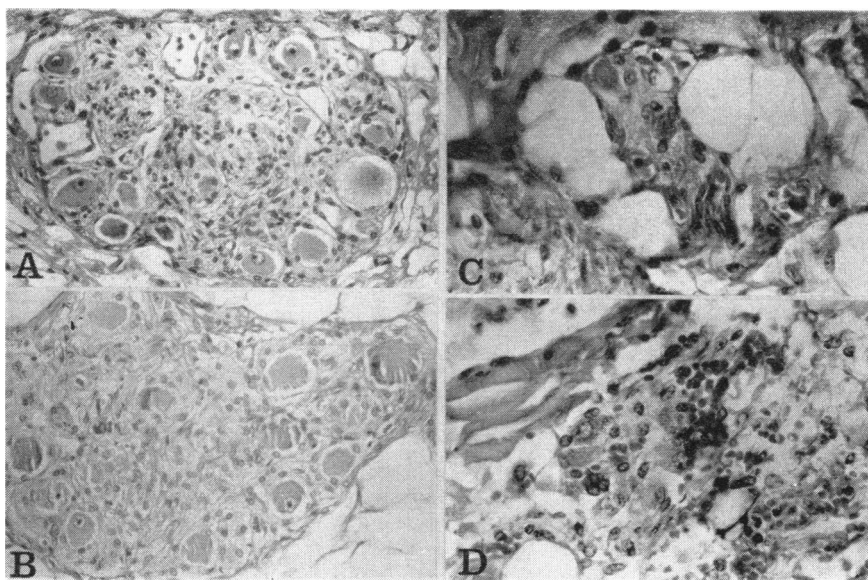
Fig. 17. Photomicrographs of typical normal *A* and *B* and pathologic cardiac ganglia *C* and *D*. Magnification in all is approximately ×90. All are from the margins of human sinus node. Those in *C* and *D* are from a child with fatal diphtheria and terminal disturbances in cardiac rhythm and conduction. Parts *C* and *D* are reproduced from *Circulation* with permission of the American Heart Association.

associated with a high incidence of arrhythmia and conduction disturbance, in which lesions of cardiac nerves may play an important role. Such lesions have been demonstrated in patients with arrhythmia due to cardiac metastasis of cancer[43] and in progressive muscular dystrophy with cardiac arrhythmia.[44] Perhaps the most striking example occurs in diphtheritic myocarditis associated with disturbances of cardiac electrophysiology,[45] for the extensive inflammation and degeneration of ganglia and other neural structures in the region of the sinus node and AV node (Figure 17) are consonant with the long-recognized clinical importance of widespread neurotoxicity in diphtheria.

Even without grossly recognizable histologic change in nerves and ganglia of the conduction system, however, certain diseases may appropriately alter their function and this in turn may cause or contribute to the onset of arrhythmia. For example, during myocardial infarction associated with an atrial arrhythmia, the region of the sinus node is regularly made ischemic, and this includes the juxtanodal ganglia.[46] Pericarditis, which is so often associated with atrial arrhythmia, regu-
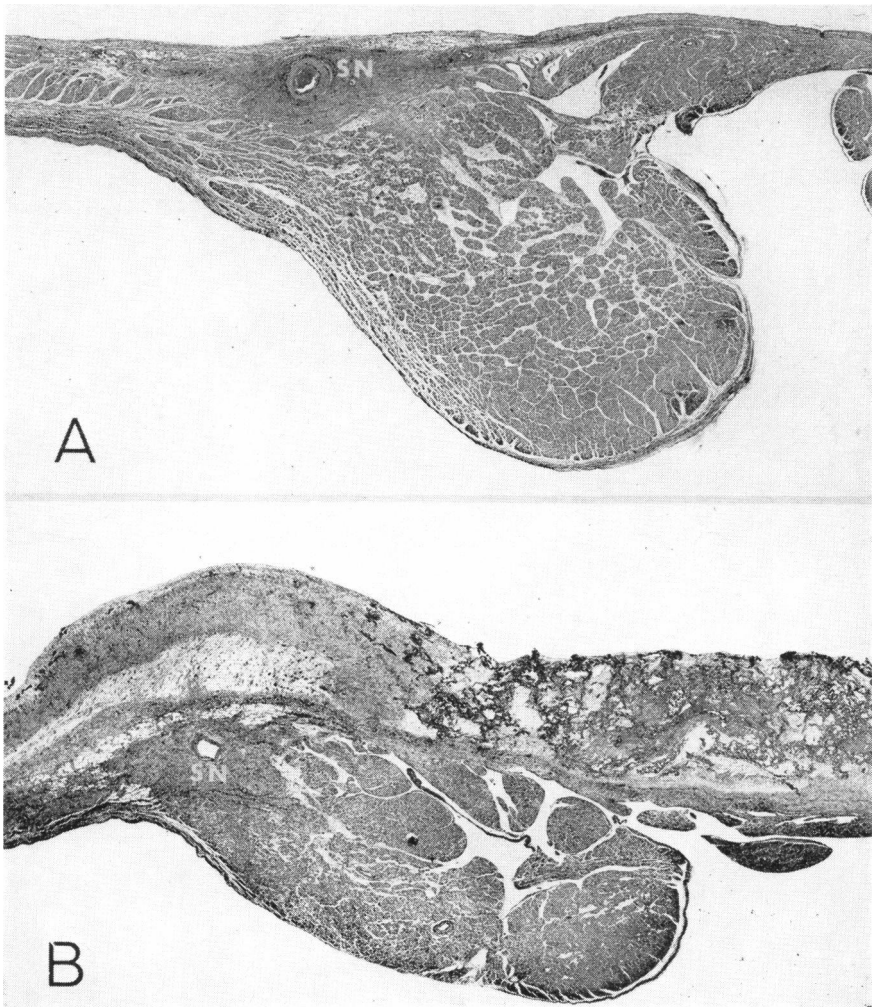
Fig. 18. Human sinus node (*SN*) is shown here normal (*A*) and with pericarditis (*B*). Extension of the inflammation directly into the sinus node is apparent, but involvement of numerous nerves is not well appreciated at this magnification ($\times 7$ in both). In each section epicardium is above, superior vena cava to the left, and free wall of right atrium to the right; the large mass of myocardium is crista terminalis.

larly involves the sinus node and its regional nerve endings and ganglia[47] (Figure 18). In acute posterior myocardial infarction there is often an associated intense sinus bradycardia and varying degrees of heart block that are frequently but not always reversible by the admission of atropine; this indicates their cholinergic mediation.[48-51] This phenomenon, which may legitimately be considered the human counterpart to the
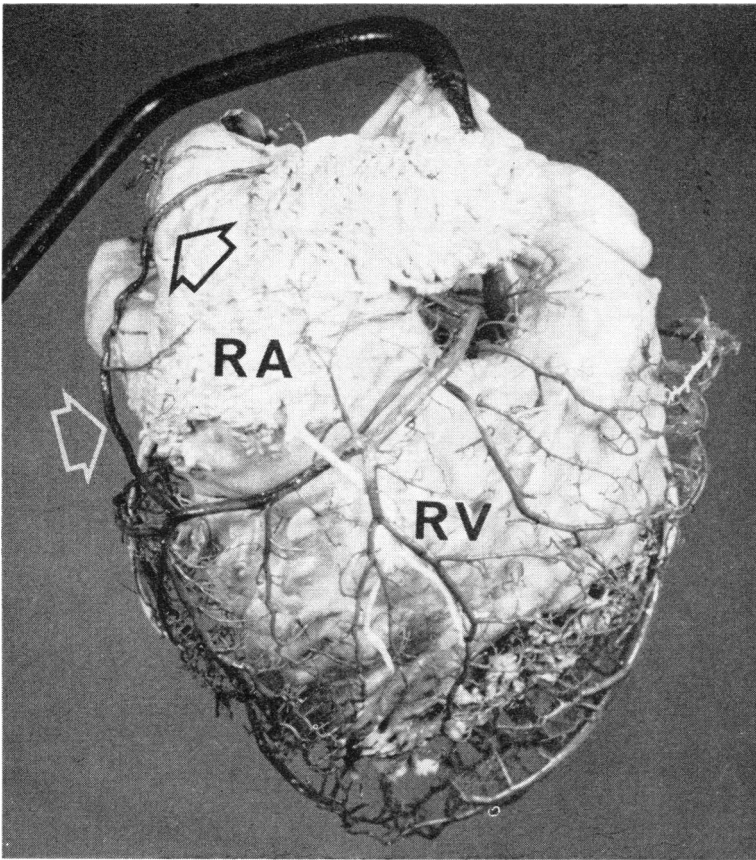
Fig. 19. This vinylite cast of a canine heart demonstrates the sinus node branch (*white arrow*) as it courses from the right coronary artery to the sinus node (*black arrow*). *RA* is right atrium and *RV* right ventricle. The sinus node artery is cannulated with a small polyethylene tube to perform the experiments described in the text. This figure is reproduced from *Anatomy of the Coronary Arteries* by T. N. James with permission of the publisher, Paul B. Hoeber.

von Bezold-Jarisch reflex seen in experimental animals, must originate within the ganglia and nerve endings that lie between the coronary sinus and AV node.[48, 49]

Cardiac Neuropharmacology Pertinent to Arryhythmias

The observations to be described here were obtained with an experimental preparation in which the sinus node of the dog was perfused directly through its nutrient artery; the cardiac innervation and blood supply remained essentially intact (Figure 19). Details of the method

have been published.[52, 53] Advantages include the possibility of direct administration of test substances into the sinus node and a small amount of neighboring atrial myocardium, without perfusion of the ventricles, other portions of the heart, the brain, or other extracardiac neurore-ceptor sites except on recirculation. Since the volumes administered are rarely more than 2 ml., recirculation is associated with approximately a 500:1 dilution of the original dose.

Direct perfusion of the intact sinus node *in vivo* permits not only the specific study of the chronotropic action of a test substance, but also the relation of this effect to neural mechanisms that influence the sinus node. The general scheme for such studies has been as follows. The primary or direct action of a test substance is determined by serial injections of increasing concentrations prepared in Ringer's solution and, when pertinent, in fresh autogenous arterial blood also. If a posi-tive chronotropic action is produced, the possibility of a vagolytic component of this action is tested by comparison of the amount of sinus acceleration before and after intranodal (10 μg./ml.) and intra-venous (1 mg./kg.) atropinization, and by comparison of the effect of the test substance on the response to intranodal acetylcholine and to stimulation of the right vagus nerve. The possibility that sinus accelera-tion is due to local release of nodal norepinephrine is tested by com-paring the effect in reserpinized and control dogs, and by observing the effect of intranodal administration of a beta-receptor blocking agent (e.g., propranolol, 10 μg./ml.) in reversing and in blocking acceleration; indirect evidence of possible release of norepinephrine is judged from the similarity of the peak and duration of acceleration to that observed with direct administration of either norepinephrine itself or of a known norepinephrine-releasing agent such as tyramine. Finally, the possibility of norepinephrine-sensitizing action (without necessarily any release of norepinephrine beyond that occurring constantly in a physiologic sense) is examined by comparison of the response to stellate stimulation and to intranodal administration of norepinephrine before and after a concentration of the test substance less than that which produced maxi-mal acceleration.

To study the mechanism of a negative chronotropic effect of a test substance, an analogous series of experiments is performed. The possi-bility of vagal mediation is tested by comparison of responses before and after intranodal and systemic atropinization, and before and after
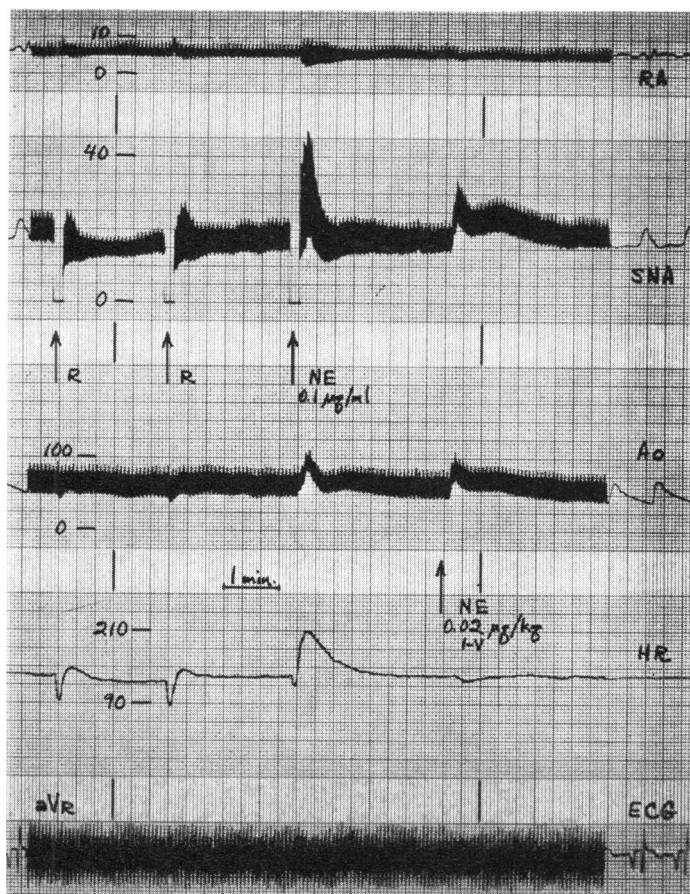
Fig. 20. This polygraph from an experiment with direct perfusion of the sinus node is typical of the recordings shown in several subsequent figures; the same scheme of labeling is used in all. The five channels from above down are right atrial pressure (*RA*), retrograde pressure in the ligated cannulated sinus node artery (*SNA*), central aortic pressure (*Ao*), a tachograph (heart rate, *HR*) derived from successive R waves of the ECG, and the *ECG*. Pressures are scaled in mm. Hg. Ligation of the sinus node artery has no significant effect on nodal function. Control injections of Ringer's solution (*R*) produce the characteristic injection bradycardia and postinjection acceleration seen in these experiments. The intranodal injection of norepinephrine (*NE*) produces immediate tachycardia, while the same amount of NE intravenously in this 10 kg. dog had only a negative chronotropic action. At this concentration NE has some pressor effect on recirculation.

cervical vagotomy. The possibility of acetylcholine sensitization (rather than accelerated release) is tested by responses to submaximal vagal stimulation of intranodal administration of acetylcholine in concentrations less than those producing maximal response. The possibility of cholinesterase inhibition is tested by comparison of responses before,
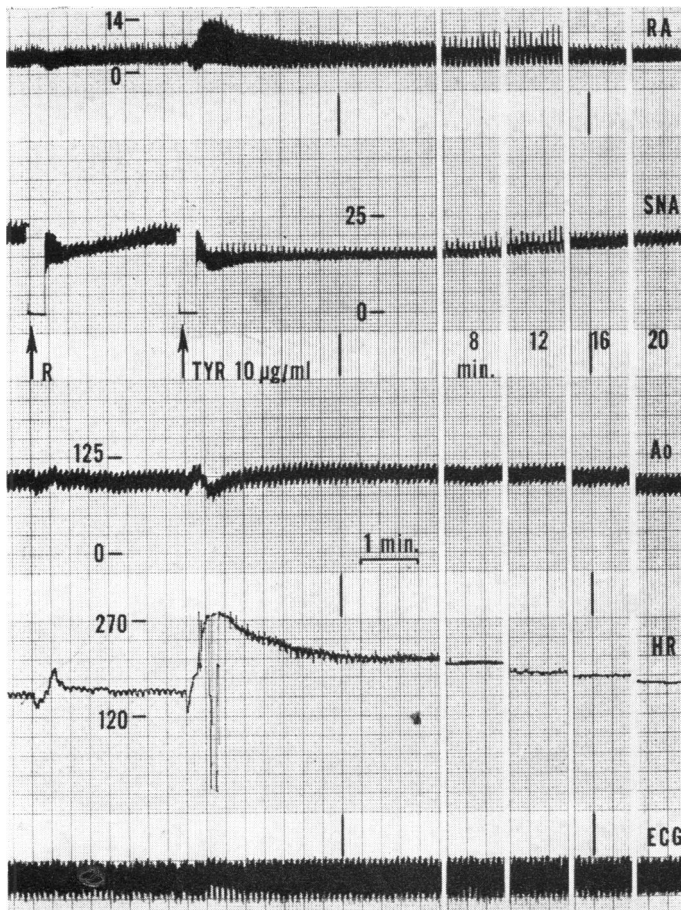
Fig. 21. In this experiment the positive chronotropic action of tyramine (*TYR*) is demonstrated, the duration being almost 20 minutes.

during and after intranodal administration of eserine (1 to 10 µg./ml.). Finally, the possibility of an antiadrenergic component of a negative chronotropic action is tested by responses to stellate stimulation or intranodal norepinephrine during the effect of the test substance.

Most of the results to be presented have been published in detail, and only those aspects pertinent to the subject of arrhythmia will be included here. Discussion of the pharmacologic studies will be divided into those dealing with adrenergic mechanisms and those dealing with cholinergic mechanisms. Some substances of course influence both mechanisms.

ADRENERGIC MECHANISMS

On direct perfusion of the sinus node, norepinephrine, epinephrine, and isoproterenol have only a positive chronotropic action, which is of immediate onset and relatively brief duration (Figure 20). In proportion to weight isoproterenol is about 10 times as potent as either of the two naturally occurring catecholamines,[54] but in concentrations producing maximal effect the duration of tachycardia is about the same. Since isoproterenol is not generally thought to occur naturally and will not enter storage sites at nerve endings into which epinephrine and norepinephrine will,[55] its greater potency may be due to the fact that all its action is direct and that no portion of that administered is stored; it thus acts in a sense as a false neurotransmitter. Ephedrine is about one tenth as potent by weight as norepinephrine or epinephrine, but its positive chronotropic effect lasts about ten times as long. Tyramine[55] acts in the sinus node principally by releasing local norepinephrine, but its duration of action is much longer than that of directly administered norepinephrine (Figure 21). In reserpinized dogs the response to tyramine can be restored by the local intranodal administration of norepinephrine or epinephrine (but not isoproterenol), but the response to stimulation of the stellate ganglion *cannot* be restored in this way. Guanethidine,[56] on direct perfusion of the sinus node, acts immediately by releasing local norepinephrine, but its duration of sinus acceleration is slightly longer than that of tyramine. Reserpine[56] has only a negative chronotropic action on direct perfusion in the sinus node; this suggests that its mechanism for nodal depletion of norepinephrine (clearly apparent hours after intramuscular administration) differs significantly from guanethidine in speed of onset of effect. Dopamine, although it has no chronotropic effect when administered intravenously, produces sinus tachycardia on direct perfusion of the sinus node.[57] Tyrosine and phenylalanine, precursors of both epinephrine and norepinephrine, have no significant chronotropic effect on direct perfusion of the sinus node.[58]

Dichloroisoproterenol[59] has a very slight positive chronotropic effect in low concentrations perfused directly into the sinus node, but in higher concentrations it effectively blocks the positive chronotropic action of locally administered norepinephrine and the sinus tachycardia from stellate stimulation. A surprising observation was the anticholinergic action of dichloroisoproterenol, apparent both against the re-
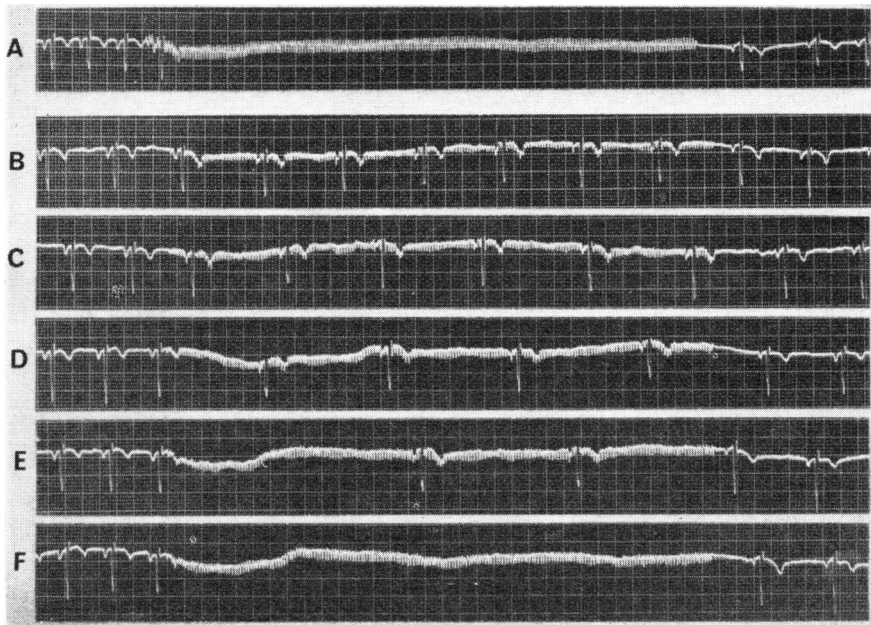
Fig. 22. These strips of electrocardiogram are aVr recorded at 25 mm./sec. and demonstrate the vagal-blocking action of intranodal pronethalol (naphthylisoproterenol), 10μg./ml. *A* illustrates the response to a vagal stimulus in the control period. The next five strips are vagal stimuli delivered at approximately three minute intervals after the pronethalol. The slower heart rate even without vagal stimulation in strips *B-E* is a direct negative chronotropic action of pronethalol. Complete recovery of the response to vagal stimulation is present in *F*. Vagal blockade by the adrenergic beta-receptor blocking agents occurs at higher concentrations than those required for adrenergic blockade.

sponse to vagal stimulation and intranodal acetylcholine.[59] Large concentrations of isoproterenol given intranodally do not have this vagal-blocking action. Pronethalol[60] and propranolol do not produce significant sinus acceleration at any concentration, but in addition to their familiar beta-receptor blocking actions they also block the response to vagal stimulation (Figure 22). The vagal-blocking action of these substances occurs at higher concentrations than their beta-receptor blocking activity, but it has been demonstrated by Wallace et al.[61] that detectable cholinergic blocking activity may be present even with concentrations conventionally employed for beta-receptor blockade. This dual autonomic blocking action of the currently popular beta-receptor blocking agents is similar to the action of quinidine (see later discussion), as is the direct negative chronotropic action after perfusion into the sinus node in high concentrations.

COMPARATIVE INHIBITION OF THE SINUS NODE BY
RIGHT VAGAL STIMULATION BEFORE AND AFTER
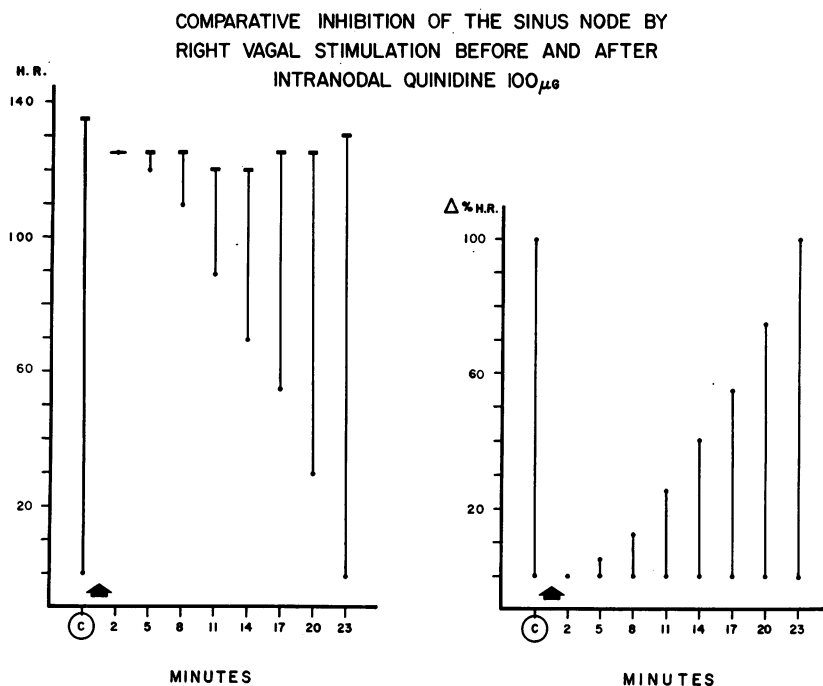INTRANODAL QUINIDINE 100μs

Fig. 23. One set of data from an experiment demonstrating anticholinergic action of intranodal quinidine is plotted here two ways: on the left the absolute change in heart rate is presented, while on the right the change in per cent is shown. Time of intranodal quinidine administration is indicated by the arrow. This and Figures 24 and 25 are reproduced from the *American Heart Journal*[67] with permission of the C. V. Mosby Co.

Acetaldehyde,[62] the principal early product of ethanol degradation in the body, has a potent positive chronotropic action on direct perfusion of the sinus node, and this is due to local release of norepinephrine. In this respect it resembles the action of tyramine or guanethidine. Ethanol, except in toxic concentrations, has no significant action after direct perfusion of the sinus node. The possible importance of a myocardial norepinephrine-releasing and depleting action of acetaldehyde in the pathogenesis of both acute and chronic cardiac effects of alcoholism deserves further study.

Digitalis may produce transient sinus acceleration on direct perfusion of the sinus node, but its more prolonged effect is one of sinus slowing.[63, 64] This action occurs in concentrations that are at or only slightly above those obtained with the therapeutic administration of digitalis. The negative chronotropic effect is not due to cholinergic

COMPARATIVE ACCELERATION OF THE SINUS NODE BY
RIGHT STELLATE STIMULATION BEFORE AND AFTER
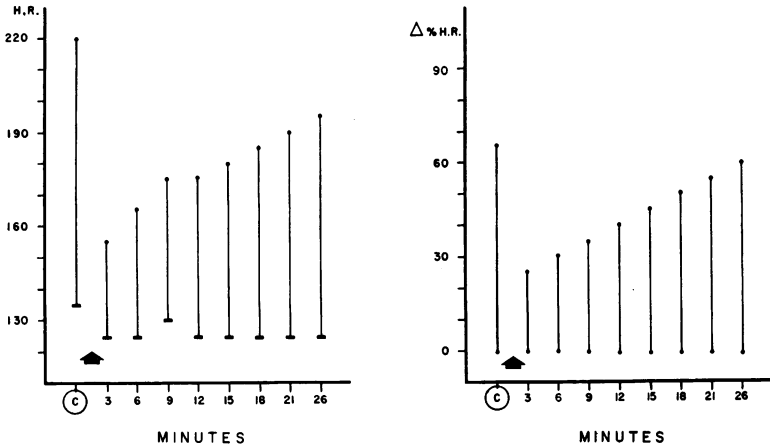INTRANODAL QUINIDINE 100 μg

Fig. 24. An experiment demonstrating antiadrenergic action of intranodal quinidine is presented here in the same way as in Figure 23. Note that in neither experiment (same dog) did the control heart rate vary significantly between neural stimulation, demonstrating that the autonomic-blocking effects occurred without significant direct chronotropic action by quinidine.

COMPARATIVE INHIBITION OF THE SINUS NODE
BY VAGAL STIMULATION AND BY INTRANODAL
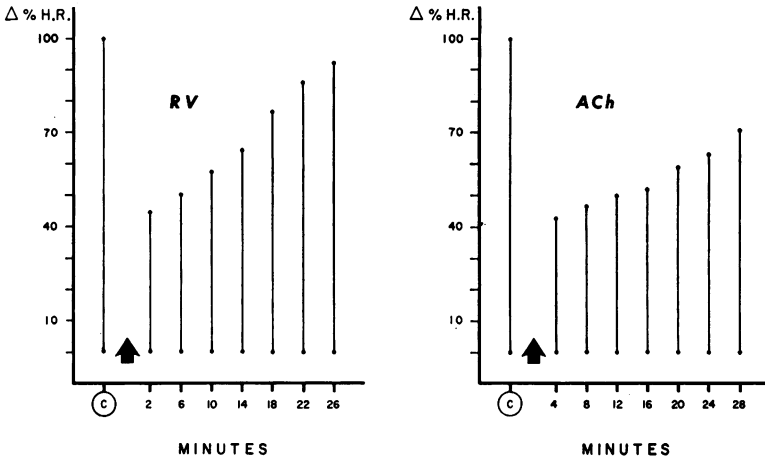ACETYLCHOLINE AFTER INTRANODAL QUINIDINE 10 μg

Fig. 25. In this experiment the anticholinergic action of intranodal quinidine is shown comparably effective against either vagal stimulation or intranodal acetylcholine, which were given alternately. The concentration of quinidine employed here is less than in Figures 23 and 24 (same dog).

mediation although, as a contributing factor, this deserves further study. There is a demonstrable antiadrenergic action by intranodal digitalis both against the effect of stellate stimulation and the direct administration of epinephrine;[65] this confirms similar observations for the action of digitalis on the AV node and the bundle of His.[66]

Quinidine (Figures 23 to 25) has both an antiadrenergic and anticholinergic action on direct perfusion of the sinus node.[67] These effects are against both neurally released and directly administered neurotransmitter substances, so that the action does not depend on inhibition of neurotransmitter release. The concentrations of quinidine with which the autonomic blocking actions occur are similar to those conventionally obtained with the clinical use of quinidine. At such concentrations there is no direct chronotropic action by intranodally administered quinidine, although higher concentrations do produce sinus bradycardia and, eventually, toxic nodal damage. In the management of clinical arrhythmia two significant points may be stressed from these findings. First, quinidine has a profound effect on the sinus node, and restoration of its normal function is the actual goal in clinical management of an arrhythmia. Second, this effect is not obvious from heart rate alone, since both the cholinergic and adrenergic blocking actions are distinctly present after concentrations of quinidine which in themselves have no direct chronotropic effect.

Nicotine has complex effects on direct perfusion of the sinus node.[68] To understand its actions it should be recalled that ganglia of the sinus node are close to it and a variable number of these are perfused by the sinus node artery both in man and the dog. The first action of intranodal nicotine is a negative effect mediated by the release of acetylcholine, due at least in part to direct action on terminals of the cholinergic nerve, since hexamethonium fails to eliminate this action while atropine does eliminate it. Following the initial slowing, which is transient, the sinus node responds to nicotine with a more prolonged acceleration, which can be abolished with propranolol and must in part be due to direct local release of norepinephrine. Hexamethonium, which failed to eliminate the negative chronotropic action of nicotine, has the distinct effect of reducing the positive chronotropic action, which is unexplained. Neither intranodal nicotine,[68] nor veratridine or veratramine,[69] produces the von Bezold-Jarisch reflex. This suggests that cardiac receptor sites for this phenomenon do not include
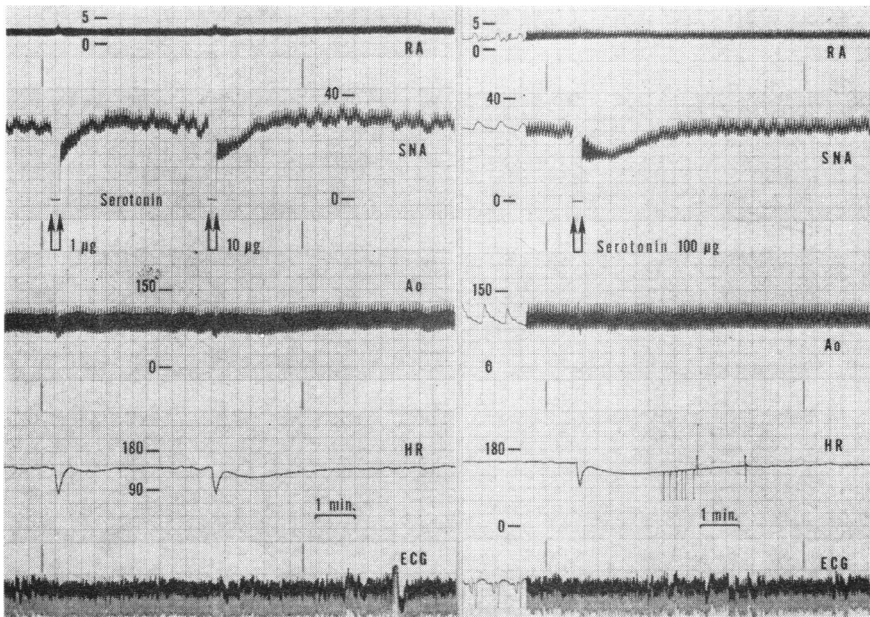
Fig. 26. The negative chronotropic action of serotonin is shown here. The onset of slowing is later than with acetylcholine or adenosine, but lasts longer. This figure is reproduced from the *Journal of Pharmacology and Experimental Therapeutics*[77] with permission of the Williams and Wilkins Co.

the region of the sinus node, which is compatible with the observations of Dawes.[70]

Bradykinin and angiotensin both release enormous amounts of catecholamines from the adrenal medulla,[71] but neither of these substances has any significant chronotropic action on direct perfusion of the sinus node.[72, 73] The reported sinus acceleration after systemic administration of angiotensin to dogs with special preparation to eliminate the secondary effect of acute aortic hypertension[74, 75] is most likely mediated by extracardiac actions or at least by actions outside the sinus node. Serotonin has many powerful extracardiac effects on the nervous system. It slows the heart when administered intravenously but accelerates the isolated heart.[76] On direct perfusion of the sinus node (Figure 26) it has only a modest but consistent slowing effect that is not due to cholinergic mediation.[77] Glucagon[78] produces sinus acceleration after direct perfusion of the sinus node, and this appears largely but not completely due to local release of norepinephrine.

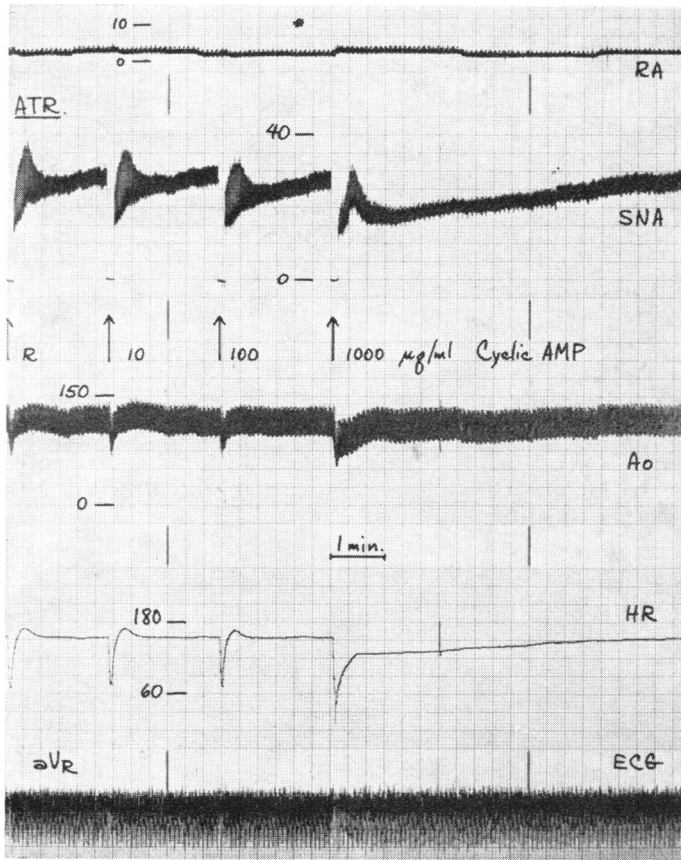Recent evidence suggests that cyclic 3′, 5′ AMP is the intracellular

Fig. 27. Characteristic negative chronotropic action of cyclic AMP is shown here, in an atropinized dog. Cyclic AMP has no significant chronotropic action at lower concentrations.

mediator for the effect of catecholamines on the myocardium.[79] However, cyclic AMP has only a negative chronotropic effect on direct perfusion of the sinus node[80] (Figure 27). The reported cardiac acceleration following its systemic administration,[81] which has been indicated as consistent with its role in mediation of responses to catecholamines, seems more likely due to secondary responses to primary extracardiac actions. Although possible it would be surprising if a normally intracellular substance such as cyclic AMP, which does not pass the cell membrane under normal circumstances, would exert the same *physiologic* action when administered extracellularly.

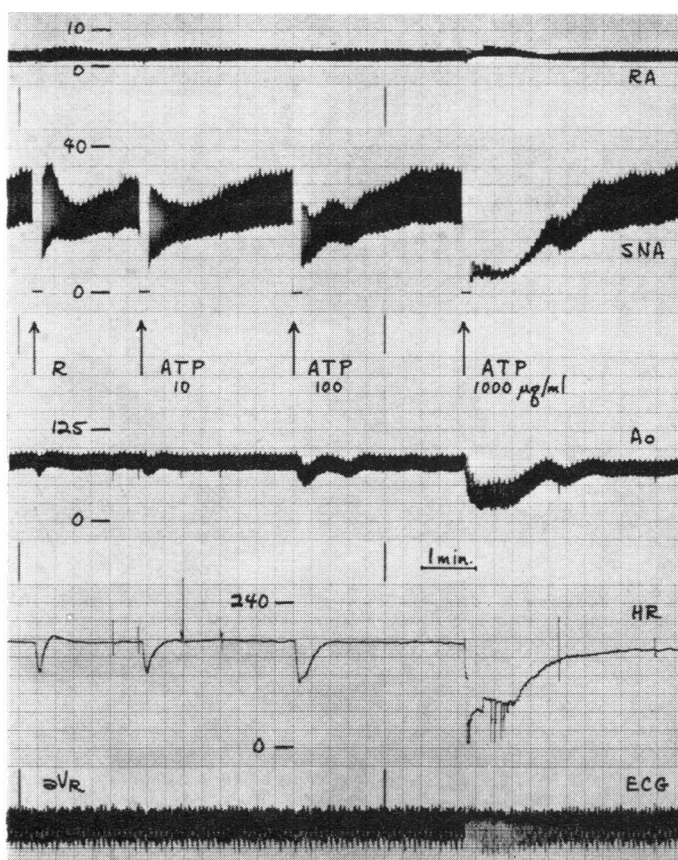In some animal species the intravenous administration of adenine

Fig. 28. ATP (adenosine triphosphate) has a greater negative chronotropic action than cyclic AMP at lower concentrations, but the duration is less.

nucleotides produces a negative chronotropic effect due to vagal mediation.[82] On direct perfusion of the canine sinus node most adenine nucleotides and closely related substances have only a negative chronotropic action without a cholinergic component[80] (Figure 28). One exception is guanosine,[79] which produces sinus acceleration unaffected by beta-receptor blockade and is most likely due to a direct effect on cells of the sinus node. Adenosine and related nucleosides do not normally contact the external surface of the myocardial cell, but during ischemia these substances are released from the myocardial cells[83] in physiologically significant concentrations and must have an influence at least on directly adjacent cells. The negative chronotropic action of adenosine (Figure 29) and its nucleotides on direct perfusion
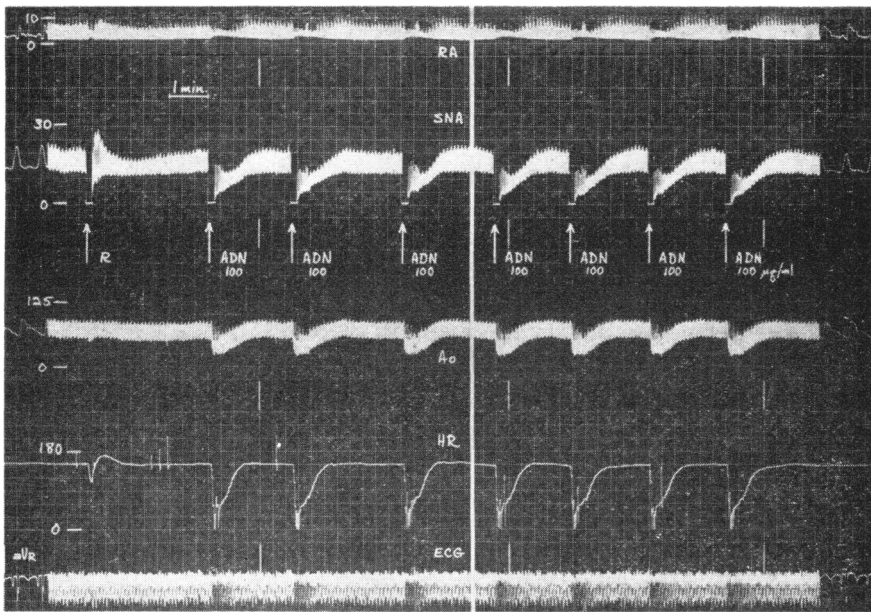
Fig. 29. Adenosine (ADN) has about the same effect as ATP on direct perfusion of the sinus node, indicating the phosphate groups (alone or cycled) are unnecessary for its action. There is no tachyphylaxis, and repeated injections produce the same effect.

of the sinus node may have a clinical counterpart in the sinus brady-cardia observed during some myocardial infarctions, particularly when this is not reversible with atropine. Similarly, the negative chronotropic action following intranodal administration of certain amino acids that are normally intracellular,[58] such as glutamic and aspartic acid, may also play a role in such bradycardias.

CHOLINERGIC MECHANISMS

A number of these mechanisms have been considered in the previous section because of logical continuity. Examples include the cholinergic mechanisms associated with actions of quinidine, the beta-receptor blocking compounds, and nicotine, as well as the noncholinergic negative chronotropic actions of digitalis, serotonin, and adenine nucleotides.

Acetylcholine[84] perfused directly into the sinus node has only a negative chronotropic action, which is progressively more marked after increasing concentrations, up to the point of complete sinus arrest (Figure 30). The negative chronotropic effect of acetylcholine
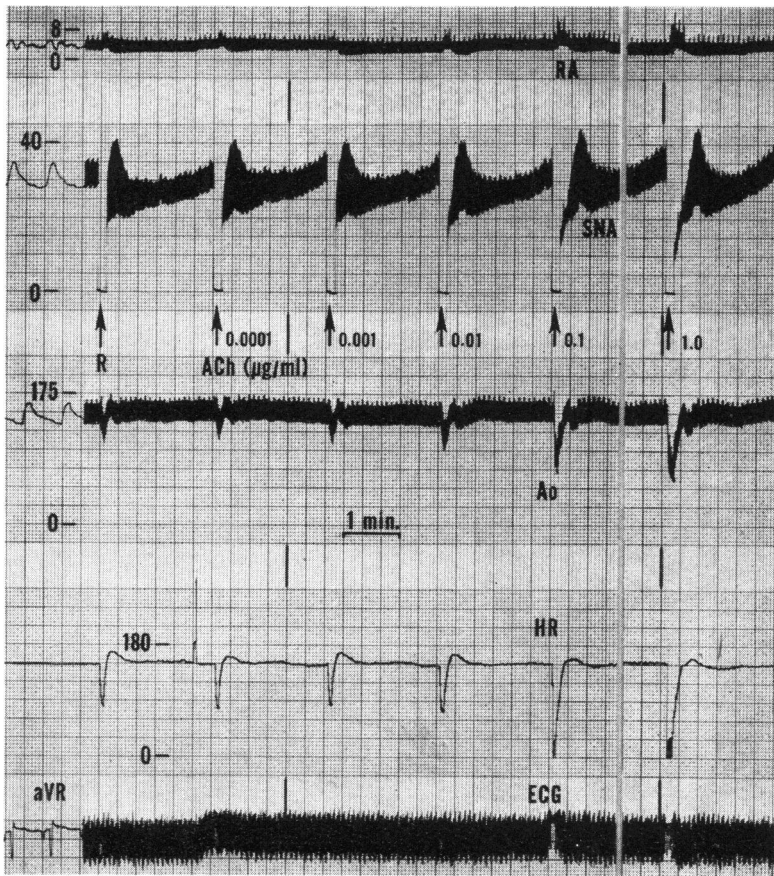
Fig. 30. Intranodal acetylcholine (ACh) has immediate but very transient effect. The higher concentrations produce sinus arrest. This and Figures 31, 33, 34, and 36 are reproduced from *Circulation Research*[84] with permission of the American Heart Association.

is immediate and extremely transient; it terminates almost as soon as the injection is completed. Its duration of action is thus briefer than that of adrenergic neurotransmitters. Very low concentrations of acetylcholine have no significant effect, and specifically do not produce sinus acceleration. Administered after either intranodal or intravenous atropinization, intranodal acetylcholine has no significant chronotropic action.

Eserine[84] produces only a negative chronotropic effect after direct perfusion of the sinus node, but this is of relatively gradual onset; it requires a minute or more to reach its maximal action (Figure 31).

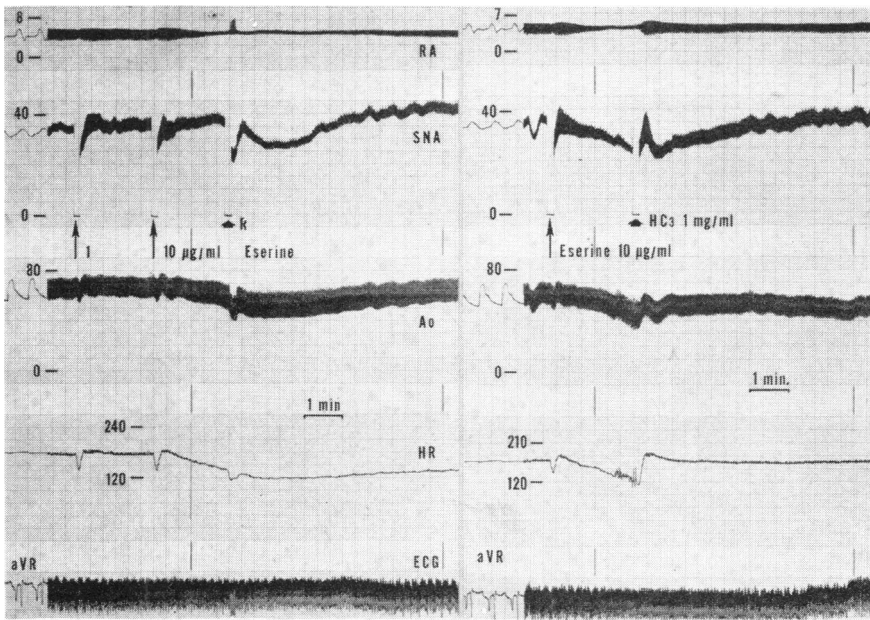Fig. 31. Eserine produces sinus bradycardia gradually but for a prolonged period. The effect is unaltered by intranodal Ringer's solution (*left panel*) but immediately reversed by intranodal hemicholinium (HC3) in the right panel. The same reversal occurs with intranodal or systemic atropinization. Since hemicholinium acts by stopping acetylcholine synthesis, its rapid action in reversing the effect of eserine indicates constant acetylcholine synthesis is required for the action of cholinesterase inhibitors on the sinus node.

Low concentrations of eserine do not produce sinus acceleration. Intranodal eserine markedly enhances the effect of even very weak vagal stimuli or very low concentrations of intranodal acetylcholine, and it prolongs their negative chronotropic action. The effect of intranodal eserine is relatively long (10 to 30 minutes, depending on the initial concentration administered), but the effect may be abruptly terminated with either intranodal or intravenous atropinization. Both eserine and acetylcholine produce atrial fibrillation after intranodal administration, especially with the higher concentrations, but this is an unpredictable effect from dog to dog and even on repeated experiments in the same dog. Atrial fibrillation so caused lasts only as long as the other direct actions of these drugs, or slightly longer, and it can be terminated and prevented by atropinization.

Production of atrial fibrillation by intranodal acetylcholine or eserine is simply a different means to the same end, since it has long been
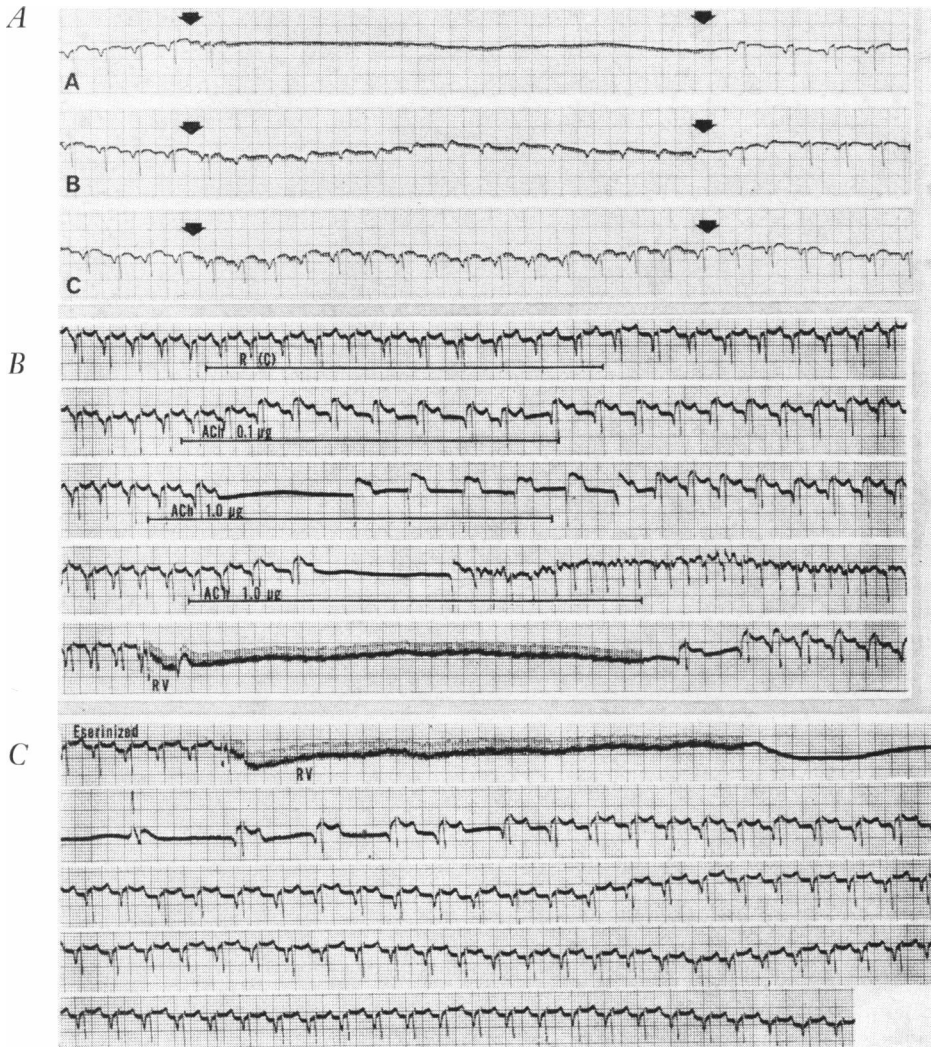
Fig. 32. These three sets of tracings illustrate various cholinergic mechanisms in the sinus node. In the upper group (*A*) a vagal stimulus is delivered before (*first trace*) and after intranodal atropine 10 μg./ml. (*second trace*) and then intravenous atropine 1 mg./kg. (*third trace*). With intranodal atropine the sinus node is selectively blocked while the AV node responds normally. In the middle group (*B*) the successive strips show response to a control injection of Ringer's solution; the response to ACh 0.1 μg./ml. (slowing, with elevation of the P-Tp segment); the response to ACh 1.0 μg./ml. (sinus arrest with AV nodal escape rhythm); atrial fibrillation produced by the same concentration of ACh on another occasion; and finally a comparative response to vagal stimulation in the same dog. In the lower group (*C*) continuous strips demonstrate the enhanced effect of a vagal stimulus after intranodal eserine in the same dog as the strips in group *B*. Paper speed is 25 mm./sec.

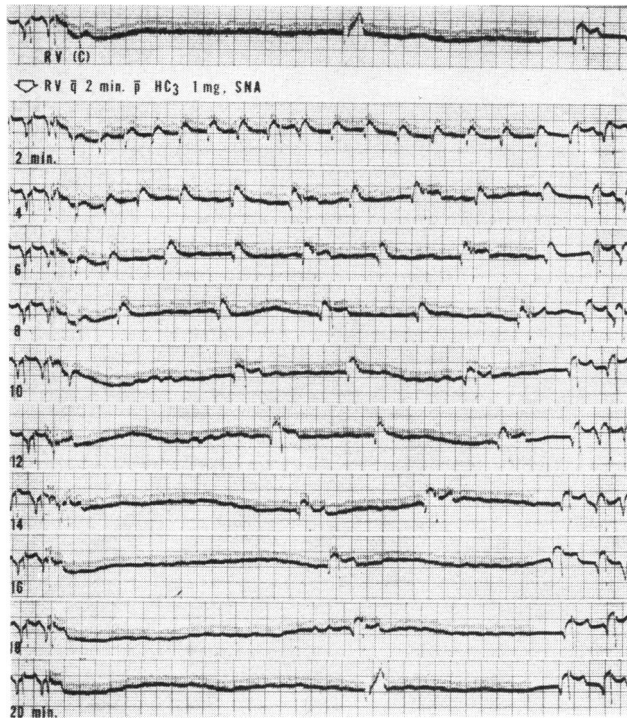Fig. 33. The effect of a vagal stimulus delivered before and serially after intranodal hemicholinium (HC3) is shown here. Note that the number of escape beats decreases as the effect of hemocholinium wanes, and the time until the first escape beat increases. P-Tp segment elevation produced by vagal stimulation here is identical to that produced by intranodal acetylcholine in Figure 32, suggesting this effect is produced during a cholinergic stimulus with or without an accompanying negative chronotropic action of acetylcholine. Paper speed is 25 mm./sec.

known that these and related substances (and vagal stimulation) enhance both the production and maintenance of experimental atrial fibrillation.[41, 42, 85-87] This effect is most likely due to the nonhomogeneous distribution of both vagal nerve endings and of cholinesterase within the atrial myocardium, so that a cholinergic stimulus, however delivered, favors the development of nonuniform excitability and recovery. Consequently such a stimulus creates the ideal situation for local reentry at many separate points and, ultimately, for fibrillation.

An analogous situation occurs in the ventricular myocardium, although the mediation there is adrenergic[88] rather than cholinergic. Stimulation of the left stellate ganglion prolongs the QT interval[89] and lowers the threshold for production of experimental ventricular fibrillation.[88] However, unlike the situation in the atria, where administration
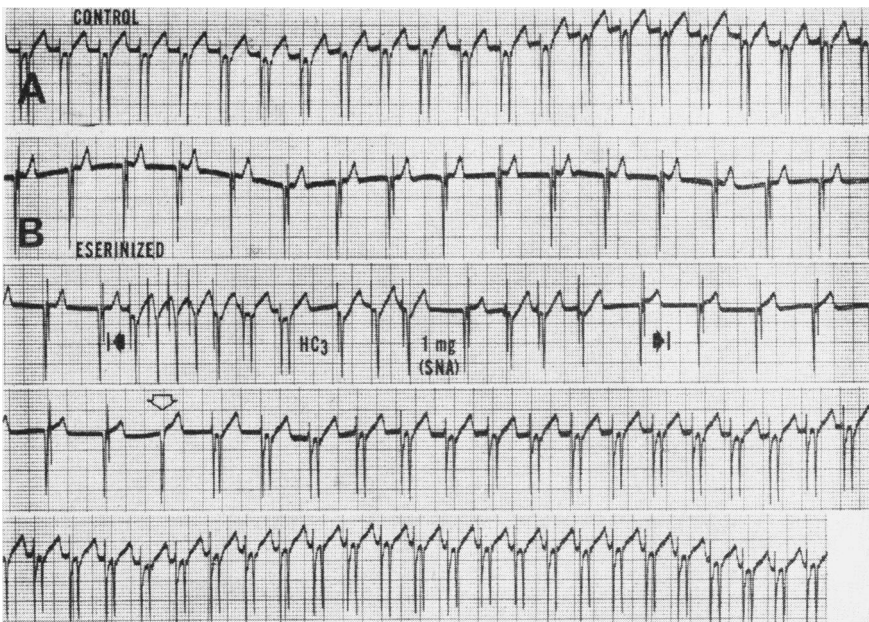
Fig. 34. This electrogram recorded from the region of the sinus node demonstrates reversal by intranodal hemicholinium of sinus arrest due to eserine. The control strip (A) is separate but the remaining strips (B) are continuous. Hemicholinium was delivered between the two black arrows and the sinus node resumes pacing at the point indicated by the open arrow. Paper speed is 25 mm./sc.

of the appropriate neurotransmitters produces the same effect, the administration of norepinephrine does not have the same effect as that from stimulation of the stellate ganglion.[88, 90, 91] Presuming that the anatomic distribution of cholinergic nerve terminals in the atria is similar to that of adrenergic nerve terminals in the ventricles—it is nonhomogeneous in both—the reason for this difference in ventricular myocardial response to neurally released and administered norepinephrine is uncertain. It may be due to more homogeneous distribution of enzymes that destroy norepinephrine in ventricular myocardium (principally catechol O-methyl transferase) than of enzymes that destroy acetylcholine in atrial myocardium (cholinesterase). An alternative possibility is a nonuniform ventricular myocardial uptake of neurally released norepinephrine but a more uniform uptake when administered directly, since recent evidence indicates uptake and storage may be more important than local degradation in terminating the effect of norepinephrine.[92]
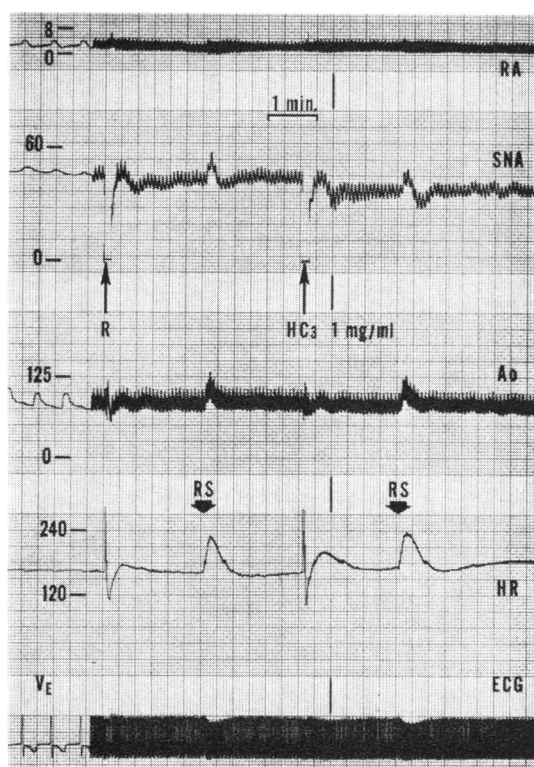
Fig. 35. Stimulation of the right stellate ganglion after intranodal hemicholinium produces the same acceleration as in the control period, indicating presence of acetylcholine is not necessary for this effect. See also Figure 36 and text for discussion.

Atropine may be used by direct perfusion of the sinus node as an adjunct to studies of certain extranodal autonomic nervous actions. Marey's reflex is a cholinergic phenomenon produced by acute aortic hypertension of any cause, and is characteristically associated with sinus bradycardia and varying degrees of AV block. The aortic hypertension after systemic administration of norepinephrine produces this reflex and has led to a misconception among some clinicians that norepinephrine has principally a negative chronotropic action. That this, of course, is not the case can be clearly demonstrated by comparing the action of norepinephrine before and after intranodal and then systemic atropinization;[93] with the former there is sinus acceleration and AV block (since the AV node is not atropinized); with the latter there is sinus acceleration with normal AV conduction. As a

Fig. 36. An electrogram recorded from the region of the sinus node is shown here during the same experiment illustrated with the p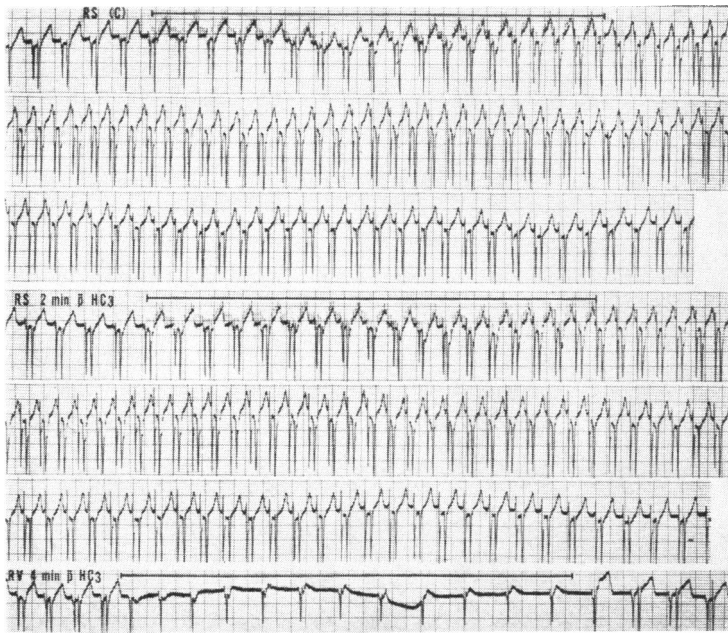olygraph in Figure 35. Note the P wave configuration is the same, excluding the possibility that the tachycardia after the second stimulus may have otherwise been ectopic in origin. The vagal stimulus response shown at the bottom demonstrates that effective vagal blockade was present even after the second stellate stimulus. Paper speed is 25 mm./sec.

teaching demonstration, the effects of right vagal stimulation before atropinization, after selective intranodal atropinization and after systemic atropinization (Figure 32), provide a clear comparison of cholinergic effects in two critical centers of the heart: the sinus node and the AV node (and the bundle of His). Participation of cholinergic discharge within the sinus node as a component of certain other reflex bradycardias (such as that early in acute hypoxemia) may also be pharmacologically "dissected" in this way.

Hemicholinium[94] inhibits acetylcholine synthesis by antagonizing the action of choline acetylase.[95] Intranodal hemicholinium does block acetylcholine synthesis (Figures 33 and 34) and is as potent as atropine in eliminating the response to stimulation of the right vagus nerve, although the effect of atropine lasts longer. Whereas atropine also blocks the effect of intranodal acetylcholine, however, hemicholinium (except for an initial inconsistent effect lasting two to four minutes)

does not block the response to intranodal administration of acetylcholine. Since it has been reported that acetylcholine is capable of releasing norepinephrine from postsynaptic adrenergic nerve terminals,[96] and that in some organs norepinephrine release after neural stimuli requires prior release of acetylcholine, experiments with direct perfusion of the sinus node were designed to test this hypothesis.[84] Hemicholinium in concentrations that completely block the response to supramaximal stimulation of the vagus have no significant effect on the response to stimulation of the stellate ganglion (Figures 35 and 36). This evidence, plus the absence of any detectable accelerating action by intranodal acetylcholine or eserine in low or high concentrations before or after atropinization, suggests that acetylcholine does *not* release physiologically significant amounts of norepinephrine in the canine sinus node, and that it is not a necessary component for such release following neural stimulation.

SUMMARY

Certain anatomic and pharmacologic observations on the innervation of the heart have been reviewed, with particular attention to their relevance to the mechanisms and management of cardiac arrhythmia. It is intended that this review will serve as a midpoint from which further considerations may become more detailed (as in experiments on the cellular membrane action potential) or, conversely, that it will cover broader interrelations (as in experiments on extracardiac factors influencing its rhythm and conduction). Molecular biology is an important new field. But cells do not behave as a homogeneous aggregate of molecules, nor do organs of the body behave as a homogeneous community of cells. It is only by critically collating the results from studies at all the various levels of biologic organization and function that we can hope to understand the normal and abnormal rhythms of the heart.

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