Circus movement in the canine atrium around the tricuspid ring during experimental atrial flutter and during reentry in vitro

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ABSTRACT    A Y-shaped lesion in the right atrium allows induction of atrial flutter in dogs. We recorded the activation sequence during this tachycardia from 96 endocardial bipolar electrodes using intracavitary electrode arrays during 12 separate episodes in three isolated perfused hearts. In each case a reentrant impulse circulated around the tricuspid valve orifice in either a clockwise or counterclockwise direction. Cutting the pathway terminated the rhythm and prevented its reinduction. There was no discrete segment of markedly slow conduction in the reentrant circuit. The tachycardia cycle length was decreased by methacholine and increased by lidocaine. Reentry was also induced in atrial tissue around the tricuspid orifice when this structure was isolated and superfused in vitro. Tachycardia cycle lengths varied from 205 to 399 msec, depending on the circumference of the ring and temperature. Induction of tachycardia by premature stimulation depended on differences in the duration of the effective refractory period among parts of the ring. Conduction velocity was relatively uniform and was slower during tachycardias than during pacing at long cycle lengths. Analysis of the response to premature stimuli that reset the tachycardia provided evidence for incomplete recovery of excitability between depolarizations during the tachycardia. Fast-response action potentials were recorded throughout the pathway and up to six to eight cell layers deep. Histologic studies showed the supravalvular lamina, a circumferential band of fibers several cell layers below the endocardial surface, to be continuous around the tricuspid orifice. Propagation through this layer best explains the conduction velocities observed in the intact heart during flutter in this preparation.


Lewis and his colleagues suggested that atrial flutter may result from circus movement around naturally occurring orifices in the atria. During brief episodes of atrial flutter induced by rapid pacing in normal dogs the activation sequence usually suggested a reentrant impulse circulating around one or both venae cavae, but the pattern of activation in one dog was more consistent with circus movement in the left atrium around the mitral valve orifice. Rosenbluth and Garcia-Ramos introduced a more stable model of atrial flutter that resulted from crushing the tissue between the superior and inferior venae cavae in dogs. Their original report as well as other studies of activation sequence in this preparation suggested that the tachycardia was due to reentrant excitation around the barrier consisting of the intercaval lesion and orifices of the superior and inferior venae cavae.

Recently we created a model of stable inducible atrial flutter in instrumented dogs by use of a more extensive right atrial lesion than that used by Rosenbluth and Garcia-Ramos. We created an intercaval lesion extending from the superior to inferior venae cavae and added a second lesion connected to the first that extended across the right atrial freewall. A rapid regular atrial tachycardia that resembled atrial flutter on the electrocardiogram was easily inducible by premature stimulation or rapid pacing. The rhythm was very stable but could be terminated easily by overdrive pacing or premature stimulation and satisfied criteria for identification of reentrant excitation. The characteristics of...
the tachycardia were consistent during successive episodes in each animal and among animals.

Activation maps constructed sequentially with use of a hand-held probe were sufficient to allow the conclusion that the reentrant impulse did not circulate around the barrier provided by the caval orifices and the right atrial incision. The impulse traveled through the intratral septum near the tricuspid orifice rather than over the left atrial freewall. However, techniques we used did not allow us to determine whether the circus path was restricted to the tricuspid ring tissues or whether it extended around the lateral end of the right atrial lesion and over the cephalad surface of the right atrium.

In the present study we have defined the location of the circus path and further characterized electrophysiologically and histologically the tissue that supports reentrant excitation in this preparation. Detailed maps of total endocardial activation sequence in blood-perfused isolated hearts demonstrated that reentry occurs in atrial tissue surrounding the tricuspid valve orifice. Conduction was relatively uniform without specific areas of markedly slower conduction. In this preparation the barrier that defines the length of the reentrant pathway is a preexisting natural obstacle. However, the surgical lesion provides an important second boundary in the sense that sustained reentry around the tricuspid orifice is readily inducible after creating the lesion but not before.

These mapping studies in intact hearts suggested the possibility that reentry could be induced in tissues of the isolated tricuspid ring. In the second part of this study we found that reentry could be induced in such a preparation in vitro. In this preparation we used both extracellular and intracellular electrodes to study impulse conduction during the induction, perpetuation, and resetting of tachycardia.

Furthermore, histologic studies of the tricuspid ring were done to determine whether a continuous circumferentially oriented fiber bundle was present. Previous studies have focused attention on radially oriented subendocardial fibers extending down the right atrial wall through the tricuspid ring and into the tricuspid valve. We demonstrate the presence of a continuous circumferentially oriented fiber bundle around the tricuspid ring that lies deep to the radially oriented fibers. Preliminary reports of these results have been made. These studies on blood-perfused isolated hearts and superfused tricuspid rings in vitro demonstrate the feasibility and usefulness of these two preparations for detailed studies of macroreentrant excitation around a fixed pathway. They also advance our knowledge of the factors important for reproducible sustained atrial flutter in instrumented dogs with a right atrial lesion. We developed this preparation to compare it with another model of atrial flutter inducible in dogs with tricuspid insufficiency and graded pulmonary artery stenosis. In that model reentrant excitation appears to be of the leading circle type, occurring around a refractory barrier.

Methods

Isolated blood perfused heart experiments. Experiments to map atrial endocardial activation sequence were performed in isolated canine hearts supported by coronary perfusion from another dog. We studied hearts from two control dogs and one dog in which a Y-shaped lesion had been made some weeks earlier to induce susceptibility to atrial flutter in vivo. For experiments on control hearts we positioned electrode arrays in the right and left atria before creating the lesion. In these experiments the activation sequence during sinus rhythm and the ability to induce a sustained regular tachycardia were both investigated before creating the Y-shaped lesion and again after creating the lesion. To induce the tachycardia we used at least five bursts of rapid suprathreshold stimuli lasting 10 sec at cycle lengths decreasing from 200 to 180, 160, 140, 130, and 120 msec or until the arrhythmia was initiated. The third heart was obtained from a dog in which the standard lesion had been made 10 weeks earlier. At that time five pairs of bipolar electrodes were implanted on the right atrial epicardium at sites near the tricuspid ring and used to induce tachycardia and record atrial activation sequence. The atrial tachycardia in this animal was similar to that described previously. The cycle length was 174 to 178 msec 9 weeks after surgery and the arrhythmia lasted at least 1 hr on each occasion when it was not interrupted by pacing.

The isolated blood-perfused hearts were prepared in the following manner. The heart was isolated from one dog (a donor dog, weight 30 to 35 kg). The coronary arteries of the isolated heart were perfused with arterial blood from another dog (the support dog, weight 30 to 35 kg). Both dogs were anesthetized with 30 mg/kg iv pentobarbital and ventilated with room air by Harvard respirators. The chest of the donor dog was opened by a midline sternotomy. Both dogs received 10,000 units heparin. A large-bore arterial perfusion catheter connected to a carotid artery of the support dog was placed in the left carotid artery of the donor dog. Arterial and coronary perfusion pressures of the heart being isolated from the donor dog were monitored with a pressure transducer (Statham, Pb23) connected to a catheter in its right carotid artery. The subclavian arteries were ligated. Another pressure transducer measured the arterial pressure of the support dog through a cannula in its femoral artery. Isolation of the donor heart was accomplished by occlusion of the aorta distal to left carotid artery and drainage of venous return from the superior and inferior venae cavae. The donor heart, perfused by arterial blood from the support dog, was removed from the thorax and transferred to the experimental apparatus. Its sinoatrial node was left intact and the atria and ventricles continued to contract. Coronary perfusion pressure of the isolated heart was determined by the arterial pressure of the support dog. The temperature of the isolated heart was monitored by a thermistor probe in the right ventricle. The temperature of a warming jacket around the arterial perfusion line was adjusted to maintain the right ventricular temperature between 37° and 39° C. Venous return from the isolated heart was collected in a large funnel and then pumped by a roller pump to a venous reservoir (a cardiotomy reservoir with a filter to remove blood clots and debris).
From the reservoir blood flowed by gravity through a warming coil into a jugular vein of the support dog.

**Electrode arrays for endocardial mapping.** Two fixed arrays of bipolar electrodes were used to record endocardial electrograms from both atria of the isolated heart. The inter-electrode distance for each electrode pair was approximately 2 mm. The distance between the centers of adjacent pairs was generally between 6 and 7 mm but ranged from 5 to 9 mm. The right atrial electrode array was made of a flexible silicone rubber material that allowed it to be deformed during insertion. It was inserted through a right ventriculotomy and then through the tricuspid ring. This electrode array had projections that extended into the right atrial appendage and the superior and inferior venae cavae (see figure 1, A and B). The left atrial electrode array was made of fiberglass in the shape of an egg. It was inserted through an incision in the left ventricular wall and then through the mitral orifice into the left atrium. Each array contained 96 bipolar electrode pairs. A total of 96 bipolar electrograms could be recorded at any one time. We recorded 96 electrograms from either the right or left atrium or 48 electrograms from each. Bipolar stimulating electrodes were sutured to the right atrial epicardium on either the free wall or aortic surface.

**Data acquisition and analysis.** A preliminary report of the system we used for data acquisition has been published. Electrograms were amplified by a bank of programmable amplifiers with band width of 10 to 1000 Hz (designed and constructed by L. Eisenberg, Rockefeller University) and then fed into an eight-bit analog-to-digital converter and multiplexing system (Preston). An automatic gain control was used periodically during the experiment to adjust the gain of each amplifier so that the amplified electrogram used the 10 V range of the analog-to-digital converter. This minimized the loss of precision caused by digitization. The sampling frequency was 4000 samples/sec for each channel. The digitized data were recorded on an Ampex high–bit rate tape recorder/reproducer with the use of a Miller code system. Data from one or two channels was monitored on a Tektronix 502 oscilloscope during the experiment.

Activation sequences during selected periods were constructed after the experiment with a PDP 11/34 computer using a UNIX operating system. Files constructed from the data recorded on tape were stored on disk (CDC 300 Mbyte). A Tektronix 4010 high-resolution graphics terminal was used to display electrograms, to permit activation times to be identified and marked, and to display activation maps on two-dimensional representations of the electrode arrays. Hard copies of these data were generated by a Tektronix Hard Copy Unit. In general the activation time was marked at the point where a predominantly biphasic electrogram crossed the baseline after the first major deflection or at the peak of the major deflection for monophasic or triphasic electrograms. Figure 1, C and D, shows how the...
two-dimensional representation of a right atrial activation map used in this study is related to the three-dimensional surface of the right atrium. The distance around the first row of electrodes around the tricuspid ring is 9.3 cm and the distance from the region labeled SAN in figure 3 to the closest point on the tricuspid ring is 2.7 cm.

Experiments with superfused isolated tricuspid rings. Healthy mongrel dogs of both sexes weighing 15 to 33 kg were anesthetized with 30 mg/kg iv pentobarbital. The heart was rapidly excised and immersed for further dissection in cold Tyrode’s solution equilibrated with 95% O2 and 5% CO2. The ring of right atrial tissue encircling the tricuspid valve (figure 2) was carefully dissected from the attached fat, ventricular tissue, left atrium, and superior portion of the right atrium. In two cases the valve was left attached; in all other cases it was removed. Full thickness of the atrial wall (1 to 2 mm) was retained except in the septum at the limbus of the fossa ovalis where tissue facing the left atrium was removed to decrease the thickness to 3 to 4 mm or less. The endocardial surface remained intact. The specimen was at least 8 mm in width at all points around the ring. In six experiments, a peninsula of pectinate muscle from the atrial freewall extending 1 cm from the lateral edge of the tricuspid ring tissue was left attached.

The entire tricuspid ring tissue from 11 dogs was studied in vitro. Ten were normal animals and one had been operated on previously and instrumented for studies on atrial flutter in vivo.7

In this animal, the cycle length of the induced atrial arrhythmias in vivo was measured the day before the in vitro study. This dog, after being anesthetized, was also ventilated with a Harvard respirator because harvesting a previously instrumented heart is more difficult. In each experiment the excised atrioventricular ring was pinned with the endocardial surface upward to a wax mold in a specially designed tissue bath. Tyrode’s solution entered in the center of the bath (in the middle of the tricuspid orifice) at 50 to 70 ml/min and was removed at the edge. The solution was continuously bubbled with 95% O2, 5% CO2 at multiple sites near the perimeter. The bath temperature was maintained at either 33° to 34°C or 37° to 38°C by prewarming the superfuse in a glass heat exchanger. The Tyrode’s solution contained in mM: NaCl 137, NaHCO3 12, NaH2PO4 1.8, MgCl2 0.5, CaCl2 2.7, dextrose 5.5, and KCl 4.0.

For the data reported here the superfuse also included norepinephrine and acetylcholine, usually at concentrations of 1.0 × 10-8 M. A higher concentration of acetylcholine (1.0 × 10-7 M) was used when the preparation did not yield sharp surface electrogams throughout its circumference or failed to sustain reentry. These neurotransmitters were used to preserve a resting potential sufficiently negative to maintain the excitability of cells in the ring after periods of quiescence. For instance, in experiments in which these agents were not present initially after the dissection in cold Tyrode’s solution the tissue demonstrated depressed excitability and conduction that gradually improved with pacing. However, transmembrane potential recordings showed marked depolarization when pacing was interrupted for only 3 to 5 sec. Longer pauses caused more depolarization with depression of excitability and conduction. This phenomena was minimized by acetylcholine and norepinephrine. Nevertheless, in five experiments in which it was attempted, reentry could be induced in the absence of these agents, and in three of these tachycardias lasted more than 15 min. The addition of these neurotransmitters tended to increase the duration of induced tachycardias when episodes were brief in their absence. In three experiments in which tachycardias where induced in the absence and presence of acetylcholine and norepinephrine (10-8 M) at the same temperature the cycle lengths were 20 to 50 msec shorter in the presence of these agents.

Stimuli were applied through Teflon-coated silver wires. A train of stimuli (S1) 2 msec in duration and suprathreshold were applied either to a trabecula in the “peninsula” of atrial free wall or to the tricuspid ring itself. A premature stimulus (S2) was delivered through the same electrodes at variable intervals after S1. S2 had the same duration as S1 but in some cases its strength was increased up to three times higher than that of S1 so as to capture at short S1-S2 intervals. A second premature stimulus (S3) was used to induce tachycardia in a few experiments; this was delivered through a second pair of stimulating electrodes, typically located 0.5 to 1.0 cm from the first. Bipolar surface electrograms were recorded simultaneously from three sites in six experiments and 10 sites in five experiments (bandwidth 50 to 500 Hz). In each case recordings were made through insulated silver electrodes placed at approximately equidistant sites around the ring.

Ten studies were conducted at 33° to 34°C to reduce metabolic rate because we suspected the thickness of the preparation might limit adequate diffusion of substrate. Four of these preparations and one other were studied at 37° to 38°C. The effect of a similar change in temperature on the cycle length of the tachycardia induced in vivo was studied in one instrumented dog that was not used for a study in vitro. The arrhythmia was induced by burst pacing through a pair of implanted atrial electrodes, and monitored with the same and four other bipolar electrodes. After the tachycardia had persisted for 30 min, the dog was anesthetized with pentobarbital, 30 mg/kg, and ventilated with a Harvard respirator. A flexible thermistor probe was inserted down the esophagus to the level of the heart. Without terminating the tachycardia, the dog then was immersed in an ice bath and the cycle length was measured as the core temperature was reduced to 33°C.

Records of transmembrane potential in the preparation in vitro were obtained through standard microelectrodes filled with 3M KCl and having resistances of 8-24 MΩ. An amplifier with high-input impedance and capacitance neutralization was used. The upstroke of the action potential was differentiated elec-

FIGURE 2. Tricuspid ring preparation. Note the landmarks of the anterior limbus of the fossa ovalis (AL), the coronary sinus ostium (CSO), and the peninsula of pectinate muscle (PM) along the free wall of the right atrium. The tricuspid valve leaflets are excised from this preparation. The wrinkling seen in the lower right (large arrow) is an artifact of the preparation’s being photographed on a flat surface; this distortion did not occur when the preparation was pinned to the convex surface of the tissue bath.
tronically to measure maximum velocity (Vmax). Calibration signals for V and dV/dt were injected between the bath and ground. Signals were displayed on a Tektronix RM565 or Tektronix 5113 oscilloscope, and the electrograms were simultaneously recorded on a four-channel Gould recorder or a Siemens Mingograf recorder at paper speeds of 2 to 200 mm/sec.

We recorded transmembrane potentials from both deep and superficial cells in five intact rings and three additional experiments in which the tricuspid ring was divided into six shorter segments. In these three experiments each of these segments was placed in a small tissue bath with the endocardial surface upward and driven at a cycle length of 1000 msec through surface electrodes. Transmembrane potentials were recorded from multiple sites that sampled the entire circumference of the tricuspid ring. At each site the microelectrode was advanced to obtain records from six to eight layers of cells. Histologic studies (see below) demonstrated that this depth was adequate to sample the cells in the circumferentially oriented fibers of the supravalvular lamina.

**Histologic studies.** At the conclusion of six experiments in vitro the preparation was fixed in 10% neutral buffered formaldehyde. The atrial ring was divided into four quadrants and labeled appropriately. For two specimens each quadrant was embedded on edge in paraffin to obtain longitudinal sections parallel to the tricuspid anulus extending the full length of the quadrant. In the remaining four specimens each quadrant was cut into four blocks of tissue and embedded flat to obtain cross sections through the ring perpendicular to the tricuspid anulus. Cross sections were also obtained from two additional hearts not used for electrophysiologic studies. For these hearts more of the right atrial and right ventricular walls and the tricuspid valve was left intact. Each specimen was cut perpendicular to the tricuspid anulus into about 20 blocks. All blocks were labeled and embedded so that the entire tricuspid anulus could be reconstructed from histologic cross sections through the atrioventricular ring.

In every case, the blocks were trimmed and further fixed for 24 hr in 10% neutral buffered formaldehyde. At least two sections were cut from each of the blocks cut perpendicular to the tricuspid anulus. The blocks embedded parallel to the tricuspid anulus were serially sectioned and every tenth section collected. All sections were 6 µm thick and were stained with hematoxylin-phloxine-saffron.

**Results**

**Condition of the isolated heart preparation and effect of the right atrial lesion on conduction in sinus rhythm.** In two experiments we recorded the atrial activation during sinus rhythm before and after the right atrial incision was made. Data recorded from 96 electrodes in one such experiment are shown in figure 3. The sinus cycle length was 390 msec. Figure 3, A, shows the concentric spread of activation from the vicinity of the sinoatrial node toward the tricuspid ring and appendage over both the free wall and aortic surfaces of the right atrium. The interval between activation of earliest and latest right atrial sites was 48 msec. Activation sequences recorded simultaneously in both atria showed a 29 msec interval between the earliest right and earliest left atrial activation (data not shown). The interval between activation of the first and last sites in the left atrium was 43 msec. The site activated last in the left atrium was located in the inferior portion of the left atrial appendage. The total atrial activation time was 72 msec.

**Figure 3, B, shows the right atrial activation sequence during sinus rhythm after creation of the**
Y-shaped incision. The presence of the lesion delayed activation of parts of the septum and the freewall surface between the transverse incision and the tricuspid ring by 10 to 30 msec. After creation of the lesion right atrial activation required 78 msec. The increase appeared to be explained by the longer conduction path rather than by any alteration of conduction velocity near the lesion or in the tricuspid ring. Activation of the left atrium was not significantly affected by the lesion.

**Atrial tachycardia induced in the isolated heart.** In the two hearts in which the Y-shaped lesion was made after positioning the electrode arrays, attempts to induce tachycardia were made both before and after creation of the lesion. Before creating the lesion we could induce only brief runs of a rapid, irregular atrial tachycardia that resembled atrial fibrillation. In one heart stimulated after only the intercaval portion of the lesion was produced, bursts of rapid pacing at cycle lengths of 160, 150, and 140 msec induced short runs of a rapid, regular atrial tachycardia with a cycle length between 110 and 120 msec. Each of the eight episodes of induced tachycardia lasted less than 1 min. Sustained tachycardia could not be induced.

After completion of the Y-shaped lesion a regular tachycardia (initial cycle length 152 msec for one and 170 msec for the other) was induced in both hearts. In the third isolated heart taken from the instrumented dog that already had the complete right atrial lesion, tachycardias with cycle lengths of 170 to 172 msec were inducible. These cycle lengths are similar to those recorded during tachycardia in vivo just before this heart was removed (178 to 180 msec). Furthermore, the activation sequences recorded from five sites during tachycardia in vivo and in the isolated heart preparation were the same. In each heart tachycardias were induced on more than one-third of trials with burst pacing at cycle lengths between 150 and 120 msec. When the atria were paced at cycle lengths of either 200 or 180 msec, single premature stimuli with coupling intervals of 120 msec also induced the tachycardia in each heart. A typical tachycardia was induced at least 20 times in each experiment. More than 50% of these tachycardias lasted longer than 2 to 3 min but all terminated spontaneously within 15 minutes. The cycle length of induced tachycardias was consistent over short periods within 5 msec, but gradually increased by 15 to 24 msec over the 4 to 5 hr recording period.

**Atrial activation sequence during tachycardias.** Maps of right atrial activation sequence obtained during 23 separate periods from 12 different episodes of regular sustained atrial tachycardia all revealed a wavefront traveling around the tricuspid orifice in either a clockwise or counterclockwise direction. Figure 4, A, shows a map of right atrial endocardial activation sequence during a clockwise tachycardia with a cycle length of 158 msec. Figure 4, B, shows the activation sequence during a counterclockwise tachycardia in the same heart but at the end of the experiment when the cycle length had increased to 180 msec. In each case an activation wavefront encircled the orifice of the tricuspid ring with a revolution time equal to the tachycardia cycle length. Figure 5 shows that the activation sequence around the tricuspid ring was the same during successive beats of tachycardia. Counterclockwise tachycardias were slightly more frequent, comprising 60% of the total. However, the direction of circus movement induced by rapid pacing in consecutive trials from a given site was not predictable.

To prove that reentrant excitation around the tricuspid ring was the mechanism for the tachycardia, we terminated the tachycardia by transecting the reentrant pathway in two experiments. An incision through the tricuspid ring was made in two steps. In the first step, an incision was made in the right atrial free wall from the transverse branch of the Y-shaped lesion to the edge of the coronary fat pad. This incision did not completely interrupt the pathway and did not terminate the tachycardia or alter the cycle length. Figure 4, B, shows one cycle of a tachycardia after the first part of the transecting incision had been made and depicts the location of this incision. The second step extended the incision caudally to the tricuspid valve and completely transected the ring of atrial tissue around the tricuspid orifice. The reentrant impulse stopped abruptly when it reached the incision, as shown in figure 5. The tachycardia stopped and could not be reinduced after this incision was made. This test proved that reentrant excitation in the tissues above the tricuspid ring was the mechanism for the tachycardia.

In addition to the primary reentrant wavefront encircling the tricuspid orifice, a secondary wavefront traveled over the superior surface of the right atrium and then toward the end of the transverse incision in both clockwise and counterclockwise tachycardias. We originally suspected that this region might comprise part of the primary reentrant pathway or an alternative to the reentrant pathway around the tricuspid ring. Figure 4 shows that during the tachycardia portions of this pathway were activated in opposite directions by wavefronts originating from opposite sides of the tricuspid orifice. Therefore the path was not part of the reentrant circuit.

Activation maps of the left atrium showed that it was also not part of the reentrant pathway. During both
clockwise and counterclockwise circus movement the left superior side of the intra-atrial septum was activated 10 to 15 msec after the right side and a broad wavefront spread laterally. The entire left atrium was activated within 35 to 40 msec which was a small portion of the tachycardia cycle length. The last activated sites were on the inferior aspect of the left atrial appendage.

Conduction of the reentrant impulse around the tricuspid ring was relatively uniform and no single part of the pathway displayed markedly slower conduction. Isochronal activation lines are more closely spaced during the tachycardia (figure 4), indicating slower conduction than during sinus rhythm (figure 3, B). However the calculated conduction velocity during tachycardia exceeded 50 cm/sec in each case (table 1).

To further evaluate conduction we observed the response to infusion of lidocaine (5 mg) or methacholine (0.3 mg) into the arterial perfusion line. Lidocaine, administered to two hearts, increased the cycle length transiently by 61 msec (159 to 220 msec) in one and by 38 msec (172 to 210 msec) in the other. Conduction was slower in all parts of the reentrant pathway. Methacholine shortened the cycle length in all three hearts by 15 to 25 msec. Conduction was faster in all parts of the pathway. Activation maps showed that neither drug altered the location of the reentrant circuit.

Reentrant tachycardia in the atrial tricuspid ring in vitro. Eleven isolated tricuspid ring preparations were studied in vitro. A rapid regular sustained tachycardia could be induced by programmed stimulation in each of the 11 experiments. Each of the following observations support the conclusion that the tachycardias result from reentrant excitation. The tricuspid ring tissue was quiescent unless stimulated. Pacing stimuli generally produced two impulses traveling in opposite directions around the ring until they collided. In contrast, after critically timed stimuli that induced the tachycardia, one of the impulses blocked allowing the other to conduct all the way around the ring in one direction. During each beat of tachycardia activation progressed in this same direction spanning the entire cycle. These points are illustrated by the examples in figure 6. In addition the rhythm could be terminated by burst pacing or properly timed premature stimuli that caused con-

FIGURE 4. A. Map of a clockwise activation sequence around the tricuspid orifice during one beat of sustained atrial flutter. The cycle depicted was arbitrarily chosen from the middle of an episode of tachycardia that has lasted more than 10 min and in which the sequence of activation for each succeeding beat of tachycardia was constant. The cycle length of the tachycardia was 158 msec and there was sequential activation of points that encircle the tricuspid orifice (wide arrow). There was centrifugal spread of activation away from the tricuspid orifice. One secondary wavefront traveled superiorly on the aortic surface (thin arrow at top) and then across the free wall of the right atrium (thin arrow at the bottom) where it encountered another wavefront spreading from the tricuspid ring toward the right atrial appendage. B. Maps of a counterclockwise activation sequence around the tricuspid orifice during 1 beat of a tachycardia later in the same experiment. A secondary wavefront spread away from the tricuspid ring in the right atrial free wall toward the appendage near the end of the transverse portion of the Y-shaped lesion. It encountered another wavefront that had emerged from the tricuspid ring area on the aortic surface and spread over the superior portion of the right atrium. When this map was recorded an additional lesion (near isochrones 50 and 60) had been made; it extended from the transverse lesion toward the tricuspid orifice. This lesion extended only part way across the reentrant pathway and did not stop the tachycardia. During the next cycle this additional lesion was extended to the tricuspid orifice so that it completely severed the reentrant pathway. The interrupted boxes around activation times in both panels indicate the location of sites from which electrograms in figure 5 were recorded. The location of the first electrode site (No. 18) is surrounded by two interrupted boxes in A.
The Perfused isolated heart experiments.

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From a heart used for both in vivo and in vitro experiments.

From a heart used for both in vivo and isolated perfused heart experiments.

FIGURE 5. The sequence of activation at nine sites during the counterclockwise tachycardia shown in figure 4, B, in a blood-perfused isolated heart. The selected recording sites encircle the tricuspid orifice in a counterclockwise sequence. The sites are identified by numbers in parentheses on the left. The cycle represented by the map in figure 4, B, is indicated here by a heavy bar at the bottom. The recording from site 18 shown on the top line is duplicated on the bottom line to illustrate the continuity of the activation sequence. Several beats of tachycardia are shown to illustrate the uniformity of the activation sequence from beat to beat. The asterisk between the electrograms recorded from sites 18 and 19 indicates the approximate time at which the incision was made between these two recording sites. That incision interrupted the reentrant excitation when the impulse reached it. The figure shows 600 msec of the rhythm and the time between vertical marks on the time line at the top line is 60 msec. Numbers indicate activation times in msec relative to a fixed but arbitrary reference time. Over each bipolar electrogram complex a vertical line has been drawn to indicate the moment when local activation occurred. Unmarked slow or low-amplitude electrograms seen in the recordings from sites 18, 19, 71, 72, and 83 reflect ventricular activation.

The cycle length of tachycardias induced in vitro. The cycle length of tachycardias induced in the isolated tricuspid ring ranged from 205 to 399 msec and depended in part on ring size and temperature, as shown in table 1. For instance, at 37° to 38°C the cycle length was 370 msec for the largest ring and 205 for the smallest. Reducing the temperature from 37° to 38°C to 33° to 34°C increased the cycle length by 36 ± 38 msec in four preparations studied at both temperature ranges.

In nine of eleven tricuspid rings at least one episode of tachycardia lasted longer than 30 minutes and in six of these the longest episode lasted more than 1 hr. In the other two, including the smallest ring and one in which the cycle length was disproportionately long for the size of the ring, (table 1) the longest tachycardias lasted only 15 and 21 min respectively. In eight experiments programmed stimulation also induced shorter episodes of tachycardia that terminated spontaneously within 30 sec. After a tachycardia stopped or had been terminated by pacing, the same tachycardia with the same cycle length could be reinduced repeatedly by...
pacing protocols similar to those that initially induced tachycardia. In nine of 11 experiments episodes of tachycardia with both clockwise and counterclockwise activation sequences were induced. In each case the cycle length of the tachycardia was similar for both directions.

Conduction around the ring during tachycardia was relatively uniform in the five experiments in which

FIGURE 6. A, A recording during a clockwise tachycardia from 10 bipolar electrodes equally spaced and arranged clockwise in a circle around the isolated tricuspid ring in vitro. B, A recording from the same 10 sites during the induction of a counterclockwise (CCW) tachycardia in the same experiment. Impulses resulting from the last two basic drive stimuli (S₁-S₂ = 300 msec) and a premature stimulus (S₁-S₂ = 180 msec) are shown at the left. Each basic drive stimulus produced two impulses that propagated in opposite directions around the ring and collided near site 9. Arrows indicate the sequence of activation and sawtooth lines indicate sites of collision of the two wavefronts. The premature stimulus (S₂) produced a response that failed to propagate in the clockwise (CW) direction, but did propagate in a CCW direction and initiated the tachycardia. In both panels the cycle length was 260 msec. Temperature, 34°C.
activation was recorded from 10 sites. Figure 6 shows similar differences between activation times at adjacent sites in all parts of the ring during clockwise and counterclockwise tachycardias in the same experiment. The greatest apparent heterogeneity occurred in one experiment in which conduction over 20% of the ring circumference occupied 35% of the cycle length.

Modes of programmed stimulation that were successful in inducing tachycardia included critically timed single or double premature stimuli after pacing at a constant drive cycle length (300 to 3000 msec) and rapid pacing at cycle lengths of 130 to 250 msec. In each case after stimuli that induced tachycardia, propagation failed in one direction but succeeded and continued in the other. The one or two sites where block occurred tended to be consistent for a given experiment but were not consistent between experiments. Critically timed single premature stimuli were usually sufficient to induce reentry when stimuli were delivered near the site of block, as shown in figure 6, B. In this example there was no evidence of a clockwise impulse even one site away from the stimulus (site 5). In other examples of induction of reentry by single premature stimuli one of the impulses conducted to one or two recording sites before blocking.

Unidirectional block of critically timed premature impulses was caused by differences in the duration of the effective refractory period between sites in circuit where propagation succeeded and sites where block occurred. Block resulted from refractoriness because less premature impulses failed to block. In four experiments we measured the range of coupling intervals that resulted in unidirectional block. These results indicated that the effective refractory period was 10 to 30 msec longer at the site of block than at the site of stimulation.

When premature stimuli were delivered on either side of the critical site, block usually would occur between the same pair of electrodes (one-tenth of the ring circumference) and at similar coupling intervals. In such cases the direction of circus movement was determined by the site of stimulation. When we did not identify the site of block and pace near it, single premature stimuli were sometimes not successful in inducing the tachycardia but other modalities were successful. These included rapid pacing or the use of two premature stimuli delivered at different sites.

The conduction velocity during the tachycardia was rate related: faster rates were associated with slower conduction. The magnitude of the effect of rate on conduction velocity and the range of cycle lengths over which it is observed is best seen during pacing at various cycle lengths. In four experiments in vitro we measured the time to conduct over 40% to 50% of the ring at various paced cycle lengths and calculated conduction velocity assuming the shortest conduction path between the two recording electrodes. Mean conduction velocity (at 33° to 34° C) decreased from 0.38 ± 0.01 to 0.26 ± 0.04 m/sec when the pacing cycle length was decreased from 600 to 260 msec. There was no significant change in conduction velocity until the cycle length was reduced below 400 msec Table 2 shows the conduction velocity at multiple cycle lengths in one representative experiment. The cycle lengths of tachycardias observed in vitro all were within the range over which rate-related changes in conduction velocity were observed.

The influence of rate on conduction velocity during tachycardia also can be seen by comparing tachycardia cycle lengths in rings of different size (table 1). There was a tendency for conduction velocity to be lower in smaller rings with shorter cycle lengths. As a result the decrease in cycle lengths with decreasing ring circumference tended to be less than expected if conduction velocity was the same. However, some of the variation in conduction velocity during tachycardia between experiments was not attributable to rate-related change in conduction velocity. For one experiment in particular the conduction velocity (0.19 m/sec) was much lower than expected when compared with other experiments.

The tachycardia cycle lengths were longer in the isolated tricuspid rings studied in vitro than observed in vivo even when variations in ring size and temperature were considered (see table 1). The largest ring studied in vitro came from a dog that had also been instrumented and studied during induced atrial flutter in vivo.7 For this dog the tachycardia cycle length in the heart in situ at 38° to 39° C was 180 msec compared

<table>
<thead>
<tr>
<th>Pacing cycle length (msec)</th>
<th>Conduction velocity (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>37</td>
</tr>
<tr>
<td>500</td>
<td>37</td>
</tr>
<tr>
<td>400</td>
<td>34</td>
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<tr>
<td>270</td>
<td>23</td>
</tr>
<tr>
<td>260</td>
<td>21</td>
</tr>
</tbody>
</table>

TABLE 2
The effect of pacing cycle length on conduction velocity (in vitro, 33° C)
with 370 msec in vitro. For each of the two conditions the cycle length was consistent within 5 msec during repeated reinductions of the same rhythm. This suggested that there was a large difference between cycle lengths in vitro and in vivo that was not due to the difference in temperature usually used for the two kinds of studies. To investigate this issue further we measured the cycle length of tachycardia in vivo at the two temperature ranges under anesthesia in another instrumented dog that was not used for study in vitro. The flutter cycle length of this dog was 147 msec at 39°C when the dog was awake and 157 msec at this temperature during the same tachycardia after sodium pentobarbital anesthesia. When the dog’s core temperature was lowered to 33°C by immersion in ice the cycle length during the same episode of tachycardia smoothly increased to 199 msec.

Evidence for incomplete recovery during the excitable gap in in vitro and isolated heart experiments. The duration of the excitable gap during tachycardias in vitro, defined as the interval during which premature stimuli could reset the tachycardia, varied from 50 to 150 msec in four experiments, with cycle lengths ranging from 259 to 350 msec. In these experiments we found evidence for incomplete recovery of excitability throughout the gap, as manifested by coupling interval–dependent conduction velocity. Any premature impulse that reset the tachycardia conducted more slowly than the normal reentrant impulse. The slowing of conduction became more marked as the coupling interval was reduced. Examples of resetting by early and late premature stimuli are shown in figure 7. More complete data from this experiment are shown in table 3. Conduction during the first poststimulus cycles varied over the entire range of premature coupling intervals.

Of particular importance is the behavior of the tachycardia is the conclusion that recovery is incomplete by the end of the excitable gap, i.e., that the normal reentrant impulse propagates through tissue with incomplete recovery. This can be shown in two ways: first, by showing slower conduction when the coupling interval is slightly shortened, and second, by showing faster conduction when the coupling interval is abruptly increased compared with that of the normal reentrant impulse. Both observations can be made after resetting of this tachycardia by single premature stimuli. The first observation can be made during the first cycle after a late premature beat that only slightly advances activation in the pathway. For instance, in figure 7, B, a premature impulse that shortens the coupling interval at site 7 by 14 msec (from 259 to 245 msec) takes 4 msec longer to complete one revolution (263 msec vs the normal cycle length of 259 msec).

The second type of evidence for incomplete recovery during the tachycardia is faster conduction after an abrupt increase in coupling interval. This can be evaluated during the second revolution of the reentrant impulse after an early premature stimulus that causes slower conduction and thus a longer revolution time during its first poststimulus cycle. For example, in figure 7, A, the revolution time during the second cycle measured at site 10 is 248 msec or 11 msec less than the normal tachycardia cycle length. Faster conduction can be attributed to the longer than normal preceding coupling interval at most sites (e.g., 285 at site 7). We measured the revolution time at site 10 because during this cycle the impulse has longer than normal coupling intervals at the greatest number of sites. Table 3 shows that conduction times during the second poststimulus cycle over shorter distances (sites 7 to 4 and 7 to 10) progressively decreased as the duration of the first poststimulus cycle (at site 7) increased to 289 msec.

With these methods we found evidence for incomplete recovery in each of the four tachycardias in which the duration of the excitable gap was measured at 33° to 34°C. In one other experiment the length of the excitable gap was not measured but the second type of evidence for incomplete recovery was provided by the response to early premature stimuli. In that case, during a tachycardia with a cycle length of 335 msec, a premature impulse that advanced activation by 70 msec near the stimulus took 365 msec to complete one revolution. During the cycle after this longer coupling interval the shortest cycle length was 308 msec, or 22 msec shorter than normal. Thus, we found evidence for incomplete recovery by the end of the gap in five tachycardias in vitro with cycle lengths up to 335 msec at 33° to 34°C. We did not evaluate whether incomplete recovery was present during the three tachycardias with the longest cycle lengths at 33° to 34°C (368 to 399 msec) or during any tachycardias at 37° to 38°C.

We also demonstrated incomplete recovery during the tachycardia in one of the isolated blood perfused heart experiments in which activation sequence was mapped after premature stimulation. This was the heart that previously had been instrumented and studied during atrial flutter in vivo. The tachycardia cycle length was 180 to 183 msec and a premature stimulus advanced activation by 55 msec. The first revolution time after the stimulus measured in the reentrant path near the site of stimulation was 221 msec. The revolution time during the next cycle was 168 msec. Again a longer than normal interval produced faster conduction than during the normal tachycardia.

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Action potential characteristics in the tricuspid ring. One advantage of this preparation of reentry in vitro is that transmembrane action potentials can be recorded from the reentrant circuit during tachycardia or pacing. We recorded transmembrane action potentials from multiple sites in five intact tricuspid ring preparations and in small portions of the ring from three other hearts. Recordings showed fast-response action potentials typical of the canine right atrium. Cells had resting membrane potentials of $-75$ to $-84$ mV and action potential amplitudes of $105$ to $115$ mV with $V_{\text{max}}$ values of $200$-$300$ V/sec when paced at long cycle lengths of $400$ to $1000$ msec. $V_{\text{max}}$ was reduced when the pacing cycle length was decreased below $400$ msec. Examples of results of pacing at cycle lengths of $400$ and $160$ msec are shown in figure 8, A and B, respec-

FIGURE 7. For legend see opposite page.
The decrease in takeoff potential at the shorter cycle length due to incomplete repolarization was accompanied by a decrease in Vmax from 200 to 100 V/sec.

During sustained circus movement the action potentials also had rapid upstrokes of 100 to 200 V/sec. We recorded an abrupt reduction of Vmax after the onset of reentry that coincided with the abrupt shortening of cycle length in one case in which an impalement was maintained during the induction of reentry (figure 9). In three of five experiments we observed clear evidence of incomplete repolarization before the next action potential during reentry, as shown in figure 9. In two other experiments it was equivocal whether, during reentry, cells attained full repolarization before the next action potential.

We observed considerable variations in the shape and duration of action potentials around the tricuspid ring. Some action potentials had a slight plateau and some did not. In one experiment we recorded action potentials from 18 endocardial sites located around the ring. The duration of action potentials measured at -60 mV averaged 140 msec but varied from 110 to 175 msec at a pacing cycle length of 400 (32.5° C). Eleven of 15 recordings obtained during tachycardia showed alternation of action potential duration ranging from 10 to 40 msec. An example from the largest ring is shown in figure 9.

We also compared action potentials recorded from superficial endocardial cells in the tricuspid ring and action potentials deep to the surface in four preparations by advancing the microelectrode progressively deeper into the tissue. We could record action potentials from cells at six to eight different depths at each site and they

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**TABLE 3**

Changes in conduction during the first two cycles after stimuli that reset a tachycardia in vitro, 34° C

<table>
<thead>
<tr>
<th>CI S2</th>
<th>CI A(7)</th>
<th>Site 4</th>
<th>Site 10</th>
<th>Site 7</th>
<th>Site 4</th>
<th>Site 10</th>
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<tbody>
<tr>
<td>259^A</td>
<td>68^A</td>
<td>176^A</td>
<td>259^A</td>
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<td>214</td>
<td>289</td>
<td>63</td>
<td>170</td>
</tr>
</tbody>
</table>

CI S2 = coupling interval (msec) from the previous activation at site 8 to the stimulus; CI A(7) = coupling interval (msec) of the premature impulse measured at site 7 during the first cycle after the stimulus. First cycle and second cycle refer to the first and second cycles after the premature stimulus. The conduction times during the first cycle from site 7 to site 7 (fifth column) in italics are the coupling intervals for the impulse during the second cycle (sixth and seventh columns).

^aIntervals and conduction times during the normal tachycardia.
FIGURE 8. Effects of pacing cycle length on takeoff potential and \(V_{\text{max}}\). Records show transmembrane potential (top) and its first time derivative (bottom). In A the cycle length is 400 msec and in B it is 160 msec. The decrease in cycle length reduced takeoff potential from \(-80\) to \(-76\) mV and \(V_{\text{max}}\) from 200 to 100 V/sec.

were not significantly different in shape, duration, or resting potential. Cells in the deeper layers demonstrated fast-response action potentials with upstroke velocities of 80 to 200 V/sec when paced at a long cycle length (1000 msec).

**Fiber orientation in the tricuspid ring tissue.** The histologic sections of the atrioventricular junction around the tricuspid ring were examined to determine whether a circumferentially oriented band of fibers, the supravalvular lamina\(^{19}\) was present in all parts of the atrial tricuspid ring tissue. We identified such a layer in every section from all parts of the ring. It is located deep to the atrial endocardial surface and just superior to the fibrous tricuspid anulus, which comprises the atrioventricular junction (figure 10). The muscle fibers of this lamina were oriented parallel to the plane of the tricuspid anulus and formed a continuous ring of 10 to 20 muscle fibers that encircled the tricuspid orifice. Between the supravalvular lamina and the endocardial surface was a layer of four to six smaller muscle fibers oriented perpendicular to the tricuspid anulus (figure 10), as described by Basset et al.\(^{10}\) This endocardial layer was separated from the supravalvular lamina by delicate fibrous tissue septa and its muscle fibers extended into the tricuspid valve leaflets. A band of muscle fibers also diverged from the supravalvular lamina to become the anterior vertical muscle bundle approximately 0.5 cm above the tricuspid anulus. The muscle fibers in the anterior vertical muscle bundle were oriented obliquely to the tricuspid anulus.

**Discussion**

We previously described an easily created stable and consistent model of atrial flutter that can be studied acutely in anesthetized animals or repeatedly over long

FIGURE 9. Simultaneous recordings of transmembrane potential and extracellular electrograms during the initiation of the tachycardia. Shown are a time line (1 sec intervals), a recording of transmembrane potential (AP), and extracellular bipolar electrograms from three sites around the tricuspid ring. Tachycardia was initiated by a single premature stimulus (S-2) following a train of stimuli at a cycle length of 900 msec. The induced tachycardia had a cycle length of 405 msec. Note the decrease in takeoff potential after the initiation of tachycardia and note the alternation in shape and duration of the action potentials during tachycardia. The chart paper speed was increased after 9 beats of tachycardia and the two time scales are indicated at the bottom. Temperature, 33.5° C. Calibration bar, upper right, indicates 100 mV.
periods of time in instrumented awake animals. In the present study we have investigated reentrant tachycardias in two different but related preparations to identify the exact location of circus movement in this model and the electrophysiologic and structural features that support stable reentry.

The location of the reentrant pathway. Atrial endocardial activation maps in the blood-perfused isolated heart demonstrated that atrial flutter always resulted from circus movement around the tricuspid orifice in either a clockwise or counterclockwise direction. The alternative possibility of reentrant excitation extending

![Image](https://example.com/image.png)

**FIGURE 10.** The histologic structure of fibers in the supravalvular lamina that surrounds the tricuspid anulus. The large central panel is a low-magnification photomicrograph of a cross section through the atrioventricular junction through the free wall of the right atrium and right ventricle. The endocardium is to the right and epicardium to the left. Right atrial (RA) tissue tapers as it approaches the atrioventricular junction. The radially oriented fibers described by Bassett et al. are located along the endocardial surface with their long axis in the plane of this section and extend into the tricuspid valve leaflet (TVL). A square on the low-power section surrounds a part of the supravalvular lamina near the atrioventricular junction that lies deep to the radially oriented fibers. This region is shown in higher power in the upper right corner. The lamina is composed of a small bundle of atrial myocytes that are cut in cross section in this view, indicating that they run parallel to the circumference of the tricuspid anulus. This bundle is located just above the fibrous atrioventricular junction and deep to the radially oriented fibers on the endocardial surface of the atrium. The long axis of the rectangular panel at the bottom is oriented parallel to the circumference of the tricuspid orifice. It demonstrates that the long axis of fibers in the supravalvular lamina is oriented parallel to the direction of circus movement around the tricuspid anulus. The stain is hematoxylin-phloxine-saffron; original magnification is ×40 in upper corner, ×100 in lower corner.
from the intra-atrial septum over the superior aspect of the right atrium and around the lateral edge of the right atrial lesion was excluded by the demonstration of a collision in this area of two wavefronts emerging from the primary reentrant circuit. The left atrium also was not involved in circus movement. The location of the path for circus movement was the same for each beat of tachycardia and for each of 12 episodes of tachycardia in three hearts.

A number of observations confirmed that the tachycardias induced after creating the Y-shaped lesion in the isolated heart preparation and in instrumented dogs were the same. In one animal the cycle length and activation sequences during tachycardia were similar in vivo and in the isolated perfused heart. In the other two isolated hearts we confirmed that the Y-shaped lesion was necessary for induction of sustained, stable tachycardia. In all isolated hearts the arrhythmia showed the same characteristics we had identified earlier for the heart in situ. These characteristics include the modes of initiation and termination by pacing, the cycle lengths, the occurrence of clockwise and counterclockwise activation patterns, and the effect of methacholine. The fact that the pattern of activation and time for total atrial activation in these experiments were similar to those previously published indicates that conduction in these isolated hearts was normal. In light of the consistency and reproducibility of the tachycardia in over 100 episodes of atrial flutter studied both in vivo and in the isolated heart we are confident that the tricuspid ring is always the site of reentrant excitation when a sustained, stable tachycardia is induced in this model of atrial flutter.

Further support for the fact that the atrial tissues surrounding the tricuspid orifice can support reentrant excitation was provided by the induction of similar tachycardias with longer cycle lengths when this tissue was isolated and studied during Tyrode’s superfusion in vitro. There was unequivocal evidence that the tachycardias in both the perfused isolated heart and the superfused isolated ring preparations were due to circus movement. During the tachycardia there was always a wavefront traveling in one direction around the tricuspid orifice. The time to complete one revolution around this structure matched the tachycardia cycle length and we could identify the location of the activation wavefront at all times during the cycle. Finally, in both preparations we applied the test that Mines identified as the best test for circus movement: we cut through the reentrant pathway and observed that the tachycardia stopped abruptly and could not be reinitiated.

Electrophysiologic and histologic factors important for reentry in the tricuspid ring. Data from both preparations indicate important features of conduction of the reentrant impulse. Conduction velocity was found to be relatively uniform with no one region accounting for a major fraction of the conduction time around the reentrant pathway. In a few of the isolated ring preparations the time between activation of consecutive pairs of electrodes equidistant around the ring varied considerably; however, the impulse may not follow a direct linear path from one electrode to the next and we are not certain that all parts of each ring were at the same point on the length-tension relationship. From the overall pattern of activation it is clear that there was no consistent area of localized slow conduction. This is of interest because previous investigators have emphasized the importance of localized regions of slow conduction as a contributing factor for reentry.

Conduction was slower during the tachycardia than at longer cycle lengths. In the isolated perfused heart preparations conduction was slower around the tricuspid ring during the tachycardia than during sinus rhythm. In the isolated ring experiments conduction velocity decreased as the cycle length was shortened below 400 msec (at 33° to 34° C) and calculated conduction velocities tended to be lower in smaller rings with shorter tachycardia cycle lengths. Similar rate-related slowing of conduction at short cycle lengths has been demonstrated during pacing in humans, usually beginning at cycle lengths longer than those typical of human atrial flutter. This rate-related slowing of conduction is multifactorial. It can be explained in part by incomplete repolarization between action potentials at short cycle lengths, as illustrated in figure 9. Other factors may include incomplete time-dependent recovery of excitatory current channels and changes in ion concentrations and ionic pump activity that effect resting potential and passive membrane properties. Regardless of the mechanisms, if the impulse conducted during the tachycardia as fast as it did during slower rhythms, stable reentry may not have been possible either in the isolated heart preparation at 38° C or in the isolated superfused ring preparation at 34° C.

Conduction of the reentrant impulse appears to be supported by fast-response action potentials. This is supported by the observation that conduction velocity exceeded 50 cm/sec and lidocaine slowed conduction in all parts of the reentrant pathway during tachycardias in the isolated heart. Furthermore, we recorded fast-response action potentials from all parts of the reentrant circuit in vitro.

The conduction velocities we observed, particularly in the isolated heart during the tachycardia, appeared
to be too fast to be explained by conduction transverse to the direction of fiber orientation as would be the case if the impulse conducted only through the subendocardial fibers that are oriented radially to the tricuspid orifice. However, we identified that a deeper band of circumferentially oriented fibers, the supravalvular lamina, is continuous around the entire tricuspid orifice. Spach et al. has demonstrated tenfold differences in conduction velocity between the longitudinal and transverse orientations in canine atrial tissue with uniform anisotropy. He demonstrated conduction at 10 cm/sec transverse to the direction of fiber orientation at long cycle lengths at 37°C. This is much slower than the conduction velocities we observed during tachycardia in the isolated hearts. We therefore believe that the supravalvular lamina supports conduction of the reentrant impulse in this preparation. We found similar fast-response action potential characteristics when recording from successive impalements as the microelectrode was passed into deeper tissue layers. In such experiments it is impossible to know which layer one is recording from or what the exact orientation of the fiber is, but we found no evidence to suggest that action potential characteristics of cells in the supravalvular lamina were different than those of more superficial cells.

The mechanism of unidirectional block during the initiation of reentry in this preparation appears to be due to transient inexcitability caused by differences in the duration of the effective refractory period at different sites in the ring. The differences we found in the duration of refractoriness in the tricuspid ring are similar to previous data from canine atria. We also found marked variation in action potential duration. It is noteworthy that in this preparation differences in the duration of refractoriness do not necessarily lead to significant differences in conduction velocity during stable tachycardia in difference parts of the ring.

An important characteristic of this preparation of reentry is that there is incomplete recovery of excitability between action potentials during the tachycardia. This is manifest by interval-dependent conduction velocity throughout the excitable gap. In this study we describe another method of demonstrating incomplete recovery: a sequence of first longer and then shorter than normal cycle lengths during the first and second cycles after a premature stimulus. The longer first cycle results from slower conduction of the premature impulse. The shorter than normal second cycle indicates that coupling intervals longer than normal for the tachycardia result in faster conduction. This criterion is only applicable when the path length does not change. Thus, under the conditions we imposed at 33°C to 34°C in isolated rings with a circumference less than 9 cm, the path length and conduction velocity observed during the tachycardia resulted in a cycle length shorter than the full recovery time in at least part of the ring.

We also demonstrated incomplete recovery by this method in one of the isolated heart preparations with a cycle length of 180 msec at 37°C to 38°C. In retrospect the same phenomenon of a shorter than normal second poststimulus cycle could be seen in a previously published example of resetting in instrumented awake dogs during this form of atrial flutter (see figure 6 in Frame et al.). Of course during reentry around the tricuspid ring in vitro or in vivo there could be complete recovery of excitability between depolarizations in very large tricuspid rings or rings with slower conduction or shorter refractory periods.

The tachycardia cycle lengths in isolated tricuspid rings were longer and conduction velocities lower at comparable temperatures than those in conscious animals or isolated hearts during flutter. Preliminary observations suggest that conduction in vitro would have been even slower without acetylcholine and norepinephrine in the Tyrode's solution. The reason for slower conduction is not entirely clear. It could be due to persistent depolarization or injury caused during the dissection or to factors such as reduced oxygen tension or elevations of extracellular potassium in deeper layers that results from diffusion limitation in a superfused preparation. Most of the experiments were performed at 33°C to 34°C to decrease the metabolic rate of the tissue. It is striking that under these conditions the electrophysiologic properties of the tissue and behavior of the reentrant circuit can remain stable during several hours of study.

The two barriers stabilize reentry in this preparation of atrial flutter. Our studies in the isolated heart demonstrate that two inexcitable barriers are needed to initiate and sustain circus movement in the intact right atrium in this preparation. Suitable electrophysiologic properties of the atrial tissue around the tricuspid orifice are not sufficient. This orifice provides one obstacle that defines the length of the reentrant path but a second barrier, the Y-shaped lesion, was necessary for sustained stable reentry, as demonstrated by the observation that flutter could not be induced before creation of the lesion. The importance of two barriers was recently hypothesized for two forms of naturally occurring flutter in a dog with diseased right atrium. Activation times recorded during flutter from 17 right atrial and seven left atrial epicardial sites in this dog suggested a wavefront circulating around a single central barrier.
consisting of the superior vena cava, the contiguous hypoplastic tissue, and possibly the inferior vena cava. In a recent reappraisal of these data, Boineau proposed that the tricuspid annulus might provide a second barrier that would help define a reentrant pathway in the dog. Unfortunately, the activation mapping was not sufficiently detailed to allow determination of the exact location of the reentrant pathway over the complex surface of the right atrium. Specifically, it is not clear whether part or all of the tricuspid ring was involved in the reentrant path and whether abnormally slow conduction in the reentrant pathway contributed to the susceptibility to reentry. By contrast, in our preparation the surgical creation of a second barrier is the necessary additional factor that facilitates stable reentry. We have evaluated the general hypothesis that two barriers are required to define all reentrant circuits. We conclude that it is too restrictive to consider only boundaries composed of inexcitable tissue. Furthermore, the need for two boundaries applies to a specific class of geometric structures. Therefore we have formulated a more general concept of the boundaries required for circus movement and the conditions under which it reduces to a requirement for two boundaries.

**General consideration of boundaries that define reentrant pathways.** We propose that two statements describe the extent and nature of boundaries required for a reentrant pathway. First, the pathway must be protected by adequate boundaries on all sides along its entire length. Second, the fundamental criterion for an adequate boundary is that it prevents short circuiting of the reentrant circuit. The extent of boundaries needed to satisfy the first requirement depends on the geometry of the tissue. In the complex three-dimensional case, a reentrant pathway in a solid mass of tissue must be totally surrounded by boundaries that prevent an impulse that diverges at any point on the reentrant pathway from returning to another point before the arrival of the reentrant impulse. On the other hand, in the case of an infinite plane of tissue with uniform conduction velocity, a single central obstacle defining the inner edge of the pathway is sufficient. However, in cases such as the right atrium in which conduction occurs on a surface of excitable tissue surrounding a central cavity, two adequate boundaries are necessary. In these cases short circuiting cannot occur through the cavity or outside the surface so the pathway has two edges on the surface that must be adequately bounded along their entire length.

We believe that adequate boundaries for reentrant pathways may be of three types. Two of these are well known and one is not. A type I boundary is a permanently inexcitable anatomic structure, such as a hole or scar. A type II boundary is an area that is temporarily inexcitable (i.e., refractory) during the tachycardia. We propose that a third type of structure can also function as an adequate boundary for a reentrant pathway. A type III boundary is an alternate pathway of excitable tissue that has a longer conduction time than the subtended portion of the reentrant pathway itself. An impulse could diverge from a reentrant pathway over this alternate path connected to another point on the reentrant circuit, but it would arrive too late to short circuit the reentrant impulse. The complete boundary along each edge of a pathway may be comprised of just one type or a combination of these different types of boundaries. When only type I and II boundaries are considered, the requirement for complete boundaries protecting both edges of the reentrant pathway would include only cases in which the excitable surface could be reduced to a single loop. However, reentry can occur when there are multiple interconnected loops. The concept of type III barriers facilitates the analysis of whether a particular loop is adequately bounded to be a pathway for reentrant excitation.

Alternate pathways may satisfy the definition of the type III boundaries either by being longer or by having slower conduction than the subtended part of the reentrant pathway. For instance, the surface of a sphere is a type III boundary when reentry occurs around the edge of a small hole on the surface. An alternate pathway over the surface of the sphere would be longer than the subtended portion of the edge of the hole. We call such a structure a geometric bulge to emphasize the greater length of every alternative path. On the other hand, a region with slow conduction can form a type III boundary if the conduction time for an impulse crossing this region is longer than that for a reentrant impulse traveling around it.

Type III boundaries are relative, not absolute, for a reentrant pathway in two ways. First, they protect reentrant pathways from alternate wavefronts arising directly from the reentrant circuit but not necessarily from impulses arising by another mechanism at another site. Appropriately timed impulses still may be able to enter and alter the reentrant circuit. Second, their existence depends on the relative path length and conduction velocity over both the reentrant and alternative pathways. Appropriate alterations of conduction velocity in either pathway could eliminate the existence of this boundary. On the other hand, progressive increases in the length of the reentrant pathway may also eliminate a type III boundary if the conduction time around the...
The reentrant pathway becomes longer than that around the alternative pathway.

Analysis of the boundaries in various models of atrial flutter. The concepts presented above may be used to determine whether specific loops in the right atrium are adequately bounded to form reentrant pathways under various conditions. Figure 11, A, shows the position of four loops we analyzed as possible reentrant pathways in the normal right atrium. Pathways one and two encircle the superior and inferior venae cavae, respectively. Past studies suggest that although these orifices may define a pathway for short nonsustained runs of tachycardia, they are not long enough to support stable reentry in the normal canine heart. However, the presence of these pathways may prevent stable reentry over longer pathways because they represent alternative pathways with shorter conduction times.

Rosenblueth and Garcia-Ramos showed that a single intercaval lesion, shown in figure 11, B, facilitates induction of a more stable form of atrial flutter. We previously presented evidence suggesting that the pathway identified as number 3 in figure 11, B, may be the reentrant circuit in that canine preparation. If this is correct then the lesion and caval orifices comprise one boundary for the pathway, and the geometric bulge of the right atrial appendage together with the tricuspid orifice comprise the other boundary. The shortest alternate path over this bulge is pathway 4, which encircles the tricuspid anulus, but we assume that the conduction time around it is longer than for pathway 3. Thus, the lateral edge of pathway 3 consists of a type I and type III boundary.

In the presence of only the intercaval lesion, pathway 4 around the tricuspid anulus is not adequately bounded because pathway 3 has a shorter conduction time. On the other hand, after creation the Y-shaped lesion (figure 11, C) the shortest alternative pathway, labeled pathway 3', is longer than pathway 4, so the tricuspid anulus is now adequately bounded. One boundary is the tricuspid orifice, but the other comprises both the Y-shaped lesion and the bulge of the right atrial appendage. In the ring preparation in vitro the second boundary is the cut that totally encircles the anular tissue and reduces it to a single loop.

In some experimental models of atrial flutter the central boundary that defines the length of a reentrant circuit is a refractory barrier rather than an anatomic obstacle. We suggest, however, that even in these preparations a complete understanding of the substrate for reentry also requires analysis of the geometric and functional factors that constitute the second boundary defining the reentrant circuit.

Models for studying specific subtypes of reentry. We believe that a more complete understanding of the responses of reentrant circuits to antiarrhythmic drugs can be achieved by characterizing and classifying these circuits based on important electrophysiologic properties of the pathway as well as the boundaries that

FIGURE 11. Schematic representation of various possible reentrant pathways in the right atrium and the influence of atrial incisions. A, Four possible pathways are shown. Pathways 1 and 2 are well defined but probably too short for sustained reentry. Pathways 3 and 4 are not well defined in the normal right atrium. B, Possible reentrant pathways in the right atrium after the intercaval lesion that interrupts pathways 1 and 2. Pathway 3 is now well defined. Pathway 4 has one boundary, the tricuspid orifice, but is not adequately bounded superiorly because pathway 3 represents a shorter alternative. C, Possible reentrant pathways after the full Y-shaped lesion (intercaval lesion and right atrial transverse lesion). The transverse lesion transects part of pathway 3, forcing an impulse to detour around the region of the crista terminalis and thus create a new, longer pathway labelled 3'. Pathway 4 is now a well-defined pathway because its revolution time is shorter than that of the alternate pathway 3'.
define it. With respect to these characteristics, the present model may be described as reentry around an anatomic barrier with no local area with markedly slower conduction. Under suitable conditions of path length and temperature there is incomplete recovery between depolarizations during the tachycardia. In this type of reentry the length of the path is fixed, but because of incomplete recovery of excitability, conduction velocity and the tachycardia cycle length are dependent on the duration of refractoriness. Therefore drugs that alter the duration of refractoriness could influence the tachycardia cycle length without changing path length. The characteristics of this type of reentry differ significantly from two other important subtypes: reentry around a fixed barrier long enough to allow complete recovery of excitability, and reentry around a functionally refractory barrier.14

This study provides information about the location of the reentrant pathway and properties of the tissue that support circus movement in a canine model of atrial flutter in vivo that can be studied either acutely under anesthesia or chronically in awake dogs.7 We have also characterized two variations of this preparation that may be useful for particular kinds of studies of reentrant excitation. In the in vivo preparation, the tachycardia can be studied repeatedly over long periods of time in a normally perfused working heart subject to intact autonomic and humoral influences. The isolated perfused heart and the superfused ring preparation allow more detailed studies of activation sequence within the reentrant pathway. The isolated perfused heart preserves the presence of alternative conduction pathways and demonstrates conduction velocities and cycle lengths similar to those observed in vivo. The superfused ring preparation allows detailed intracellular and extracellular recording during reentry from all parts of the pathway. Many of the fundamental insights into the nature of reentrant excitation by Mayer,30 Mines,9 Garrey31 and others came from studies in rings of excitable tissue studied in vitro. It is noteworthy that Mayer found evidence for rate-dependent conduction during reentry in rings from Cassiopea xamachana by observing the effect of increasing the length of the circuit. Our model differs from these early models of anatomic barrier reentry in that a natural obstacle defines the length of the pathway. Such preparations hold promise for studies relating the electrophysiologic properties in parts of the pathway to the overall behavior of the arrhythmia. Thus, each of these three variations provides particular advantages as preparations for understanding the responses of a particular subtype of reentry.

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