the binding of free G for the 5′ splice-site attack (*19*).

These data suggest that group I introns could use a nucleoside other than G in the splicing reaction. With the A:U variant (*13*), splicing-related activities were observed with the substrates r2AP and 2,6-diaminopurine ribonucleoside. In our report, another set of changes in the intron resulted in splicing activities with rP and A. Of these four substrates, the likely alternative to G, for splicing in vivo, is A. However, the variant splicing reaction with A requires high nucleoside concentrations and even then appears to proceed more slowly than the splicing of the wt precursor with G. It may be possible to introduce additional changes so that A will work in vivo. If so, it can be argued that either the specificity for G is arbitrary but difficult to change or is imposed for reasons not directly related to the splicing reaction. If, however, changes cannot improve A-dependent splicing, it may be that G works best and thus was selected on the basis of optimal splicing activity.

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15. Cleavage in the absence of nucleoside is due to hydrolysis at the 5′ splice site and by hydrolysis at the 3′ splice site followed by circularization to the 5′ splice site (*8*). For the C:G variant, we observed an enhanced rate of nucleoside-independent 5′ splice-site cleavage that was pH-dependent. This increased rate of hydrolysis at the 5′ splice site may also account for the production of ligated exons in the triple mutant (G264C:C311G:G414A) in the absence of added adenosine (Fig. 4).
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18. The velocities (ν) of the reactions were determined [calculated as the fraction of Sca I–runoff (*14*) cleaved per minute during the initial phase of the reaction] under the same conditions as described for Fig. 4, but with varying nucleoside concentrations. The K_m and V_{max} were obtained from Eadie-Hofstee plots (ν versus $\nu/[S]$; $[S]$ is the nucleoside concentration). For the C:G variant, the V^{rP}_{max} (± SE) = 0.18 (±0.03) min^{-1}, K^{rP}_m = 0.3 (±0.05) mM, V^{A}_{max} = 0.17 (±0.05) min^{-1}, and K^{A}_m = 1.2 (±0.2) mM. For the wt sequence with G, V_{max} = 0.8 (±0.1) min^{-1} and K_m = 0.01 (±0.005) mM.
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Dynamic Tracking of Cardiac Vulnerability by Complex Demodulation of the T Wave

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A link is found between T wave alternans and vulnerability to ventricular fibrillation, and a new approach is provided for quantification of susceptibility to malignant arrhythmias. Complex demodulation reveals that alternation of the electrocardiogram is concentrated during the first half of the T wave, coinciding with the vulnerable period of the cardiac cycle. During myocardial ischemia and reperfusion, there are marked increases in the degree of T wave alternans that parallel the established time course of changes in vulnerability. The influence of the sympathetic nervous system in arrhythmogenesis is also accurately detected. Ultimately, complex demodulation of the electrocardiogram could provide a technique for identification and management of individuals at risk for sudden cardiac death.

SUDDEN CARDIAC DEATH, WHICH claims over 350,000 lives annually in the United States, results from abrupt disruption of heart rhythm, primarily in the form of ventricular fibrillation. Death is due not to extensive cardiac injury but rather to transient neural triggers that impinge on the electrically unstable heart (*1–3*). Identification of individuals at risk for sudden cardiac death remains a major objective in cardiology. Programmed cardiac electrical stimulation provides quantitative information but introduces the hazard of inadvertent induction of ventricular fibrillation. Quantification of T wave alternans is a promising approach because of its intrinsic safety and because of the consistent occurrence of T wave alternans before fibrillation under diverse conditions including coronary artery occlusion, hypothermia, Prinzmetal's vasospastic angina, and the long Q-T syndrome (*4–7*). Smith and co-workers (*4*) and Adam and co-workers (*8*) showed a correlation between fluctuations in overall energy of the T wave and the ventricular fibrillation threshold during coronary artery occlusion and hypothermia in dogs.

We studied a total of 16 adult mongrel dogs (20 to 30 kg) of either sex in accordance with the standards of the scientific community (*9, 10*). The animals were premedicated with morphine sulfate (2 mg per kilogram of body weight, subcutaneously) and anesthetized with α-chloralose (150 mg kg^{-1}, in-

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travenously), with supplemental doses of α-chloralose (600 mg in 60 ml of saline) administered as required. A left thoracotomy was performed at the fourth intercostal space. A Doppler flow probe was placed around the left anterior descending (LAD) coronary artery, and we performed occlusions using a 2-0 silk snare. Aortic blood pressure was measured with a Gould-Statham P50 pressure transducer. The electrocardiogram (ECG) was obtained with a 7 French USCI quadripolar catheter with an interelectrode distance of 10 mm and electrode width of 2 mm. The tip of the catheter was positioned in the apex of the left ventricle through a carotid artery. We obtained bipolar ECGs by using a needle electrode placed transcutaneously in the lower left hip region as the positive pole and the proximal electrode of the catheter as the negative pole. A pigtail pressure catheter was positioned to monitor left ventricular (LV) blood pressure. We analyzed the area under the LV pressure pulse of successive beats using the technique of complex demodulation. No evidence of mechanical alternans was found.

The electrocardiographic and hemodynamic data were continuously recorded on a Thorn EMI FM tape recorder (45 to 50 dB signal-to-noise ratio, bandwidth of each channel 0 to 625 Hz). We monitored arterial blood pH, the partial pressure of CO_2, and the partial pressure of O_2 by using an Instrumentation Laboratory 1304 blood gas analyzer and maintained values within physiologic ranges by adjusting the ventilation parameters of a Harvard respirator. Bilateral stellectomy to interrupt sympathetic neural input to the heart was accomplished by complete removal of the right stellate ganglion through the right second interspace and by sectioning of the preganglionic fibers and the caudal end of the left ganglion through the left thoracotomy (*11*). The ansae subclavia were left intact to permit delivery to the nerves of electrical stimuli of 1.5 to 2 mA of 5-ms duration each at a frequency of 10 Hz with a Grass S44 stimulator and SIU7 stimulus isolation unit.

At the end of each experiment, the recorded data were low-pass filtered to limit the signal bandwidth to 50 Hz before digitization at 500 samples per second with a Compaq 386 computer equipped with a Metrabyte DAS-20 analog-to-digital conversion board. The periods from 60 to 290 ms after the R wave of each successive beat, indexed by n, were divided into bins 10 ms wide. To derive a single time series, $X(n)$, the area between the ECG and the isoelectric baseline was computed for each 10-ms interval. Then N successive beats from control through release were sequenced into a time series for each of the 23 10-ms bins: $[X(n), n = 1, 2, \ldots N]$. The R-R interval was used to sort out and remove premature beats that could introduce artifactual spikes. A sixteenth-order Butterworth filter (*12*) was used for both detrending and demodulating to remove the large low-frequency variations in T wave area that occur during occlusion and to leave a cleaner signal for spectral estimation.

To obtain estimates of the magnitude of beat-to-beat alternation in the amplitude of each of these time series, we used the method of complex demodulation (*13, 14*), a type of harmonic analysis that provides a continuous measure of an oscillation with slowly changing amplitude and phase. It detects features that might be missed or misrepresented by standard Fourier spectral analysis methods, which assume that the data are produced by a stationary process. Although alternans by definition is a periodic alternation in T wave amplitude, the magnitude of alternans changes slowly during occlusion and more rapidly upon release. Thus, the magnitude is quasi-periodic and must be represented by a sinusoid with slowly varying amplitude, $A(n)$, and phase, $\phi(n)$

$$X(n) = A(n) \cos [2\pi f_0 n + \phi(n)] \quad (1)$$

where f_0 represents the frequency of alternation in cycles per second. This frequency is then half of the paced heart rate. To use the method of complex demodulation, we multiplied the time series by two times a complex exponential at the alternans frequency

$$Y_1(n) = A(n) \exp[i\phi(n)] + A(n) \exp[i2\pi\ 2f_0 n + i\phi(n)] \quad (2)$$

We then filtered the result to retain only the low-frequency term

$$Y_2(n) = A(n) \exp[i\phi(n)] \quad (3)$$

Thus, the amplitude and phase of the alternans is found from the filtered signal as follows

$$A(n) = |Y_2(n)| \quad (4)$$

$$\phi(n) = \arctan \left\{ \frac{Im[Y_2(n)]}{Re[Y_2(n)]} \right\} \quad (5)$$

where *Im* and *Re* refer to the imaginary and real parts of Y_2.

We tested the effects of LAD coronary artery occlusion and reperfusion on T wave alternans before and after sympathetic denervation and stimulation. Baseline data were obtained for 4 min, and the artery was occluded for 8 min, followed by an abrupt release-reperfusion and a 30-min rest period. A constant heart rate was maintained by atrial pacing at 150 beats per minute during assessment of the magnitude of alternans. The first, or "preconditioning," occlusion-release sequence is associated with a high degree of variability (*15*). In eight dogs, the preconditioning occlusion was followed by a control occlusion with nerves intact. We repeated the occlusion-release sequence after stellate ganglion ablation. Finally, the left stellate ganglion was stimulated 2 to 3 min before occlusion, during the second and fifth minutes of occlusion, and during reperfusion. In the second group of eight dogs, we changed the order of interventions to rule out sequence-related error by omitting the

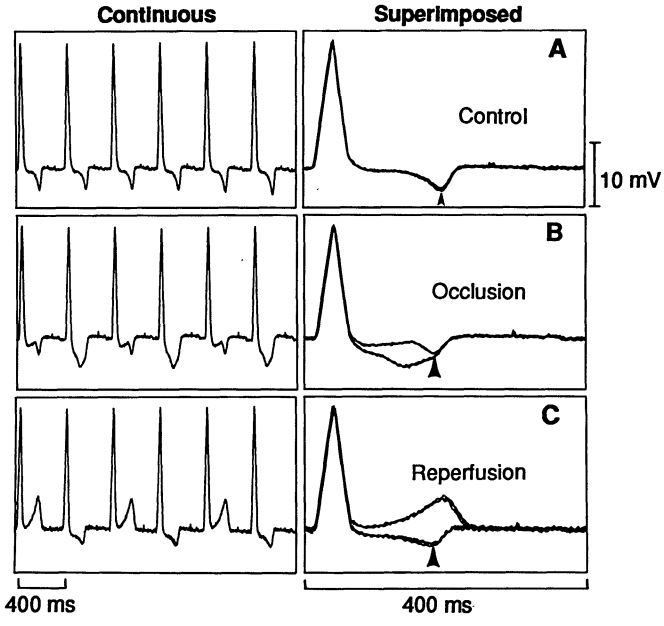
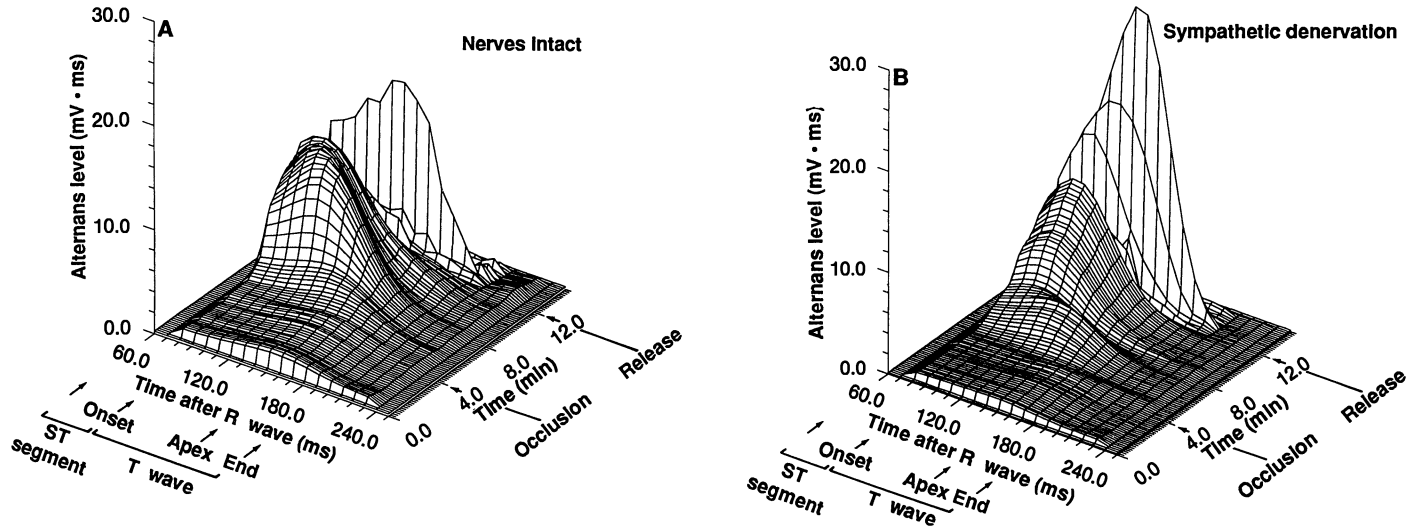


Fig. 1. ECG recorded within the left ventricle before, during, and after coronary artery occlusion in a single representative animal. The pattern shown was observed in all animals studied. Right panels show superimposition of six successive beats. Before occlusion (**A**), the T waves of each succeeding beat were uniform (arrowhead designates apex of T wave). After 4 min of coronary artery occlusion (**B**), there was marked alternation of the first half of the T wave, coinciding with the vulnerable period of the cardiac cycle. The second half of the T wave remained uniform. After release of the occlusion (**C**), alternans was bidirectional, with T waves alternately inscribed above and below the isoelectric line.



occlusion with intact nerves.

Both coronary artery occlusion and reperfusion resulted in significant increases in the magnitude of beat-to-beat alternation in T wave amplitude (Figs. 1 and 2A). The increase in alternans was evident within 2 to 3 min of occlusion and progressed until the occlusion was terminated at 8 min. Upon reperfusion, there was an abrupt increase in alternans, which lasted less than 1 min (Fig. 2A). The pattern of alternation during reperfusion was bidirectional, with T waves occurring alternately above and below the isoelectric line (Fig. 1).

The time course of onset and offset of T wave alternans during the occlusion-release sequence coincides with the spontaneous appearance of malignant tachyarrhythmias, including ventricular fibrillation (*1*) (Fig. 3). Alternans is marked, although of short duration, during reperfusion. This transient period of heightened vulnerability to fibrillation is thought to be due to liberation of washout products of cellular ischemia (*16, 17*). The differing mechanisms responsible for vulnerability during occlusion and reperfusion may account for the contrasting alternation patterns in T wave morphology.

Our studies demonstrate that the sympathetic nervous system exerts a prominent effect on T wave alternans, a finding that is consistent with its established arrhythmogenic influence (*2, 3, 18–20*). Stellectomy (Fig. 2B) reduced alternans during the early phase of occlusion [from 15.8 ± 6.6 mV · ms at 4 min during control (Fig. 2A) to 4.7 ± 1.0 mV · ms (mean ± SEM, $P < 0.05$)], coinciding with the time during which neural activity is high in intact animals (*18*). However, later in the occlusion extraadrenergic factors may play a role.

Sympathetic neural influences during the reperfusion phase also appear to be reliably tracked by the present techniques. We observed that stellate ganglion ablation increased T wave alternans during reperfusion [from 19.8 ± 3.0 to 29.8 ± 3.3 mV · ms ($P < 0.02$)]. This concurs with the results of a previous study indicating that stellectomy enhances reperfusion-induced vulnerability to fibrillation (*18*). The probable basis for the enhanced vulnerability and alternans is that reduction of sympathetic tone to the coronary vasculature results in enhanced liberation of ischemic products, leading to increased cardiac electrical instability and fibrillation. Stellate ganglion stimulation restored the magnitude of alternans to a value that was not statistically different from predenervation levels. This is presumed to be due to the beneficial effects of maintaining

Fig. 2. Surface-plot display derived by complex demodulation of the T wave of the electrocardiogram before, during, and after coronary artery occlusion (**A**) in eight dogs with intact cardiac innervation; (**B**) after bilateral stellectomy (sympathetic denervation) in six dogs; and (**C**) during four 30-s stimulations of the ansae subclavia of the decentralized left stellate ganglion (sympathetic stimulation) in 11 dogs. (The first stimulation was in the preocclusion period.)

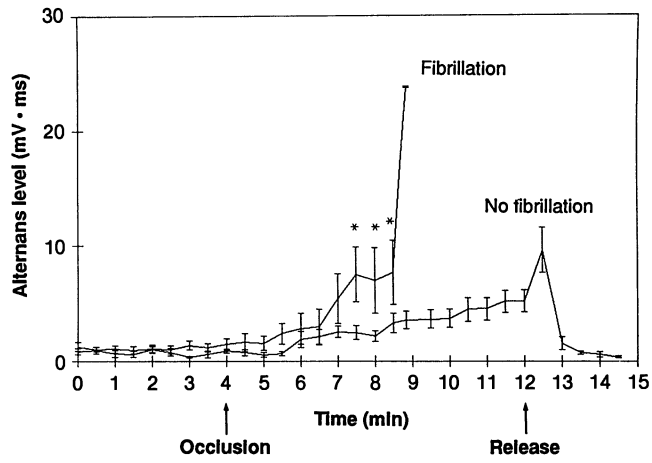


Fig. 3. Correlation between the occurrence of spontaneous ventricular fibrillation and T wave alternans in ten dogs. Dogs that fibrillated exhibited a rapid rise in alternans within the first 3 or 4 min of occlusion, and this change was significantly more marked than that observed in animals that survived the entire occlusion-release sequence. * = $P < 0.001$; values are mean ± SEM. We analyzed the results using a one-way analysis of variance with Scheffé correction for multiple comparisons. In both groups, the control values did not differ significantly from the normal distribution according to the Kolmogorov-Smirnov test.

coronary tone, thereby ameliorating the effects of reactive hyperemia (*11, 17*).

The link between alternans and vulnerability is underscored by the finding that alternans coincides with the established timing of the vulnerable period in the cardiac cycle (*21–23*). Superimposition of successive beats indicates that alternation is restricted to the first half of the T wave (Fig. 1, right panels). This relation remained constant in all animals studied under the changing conditions of sympathetic nervous system stimulation or denervation (Fig. 2, A to C).

The precise electrophysiologic basis for the correspondence between alternans and vulnerability remains to be determined. One possibility is that alternans represents increased dispersion of repolarization, which is most marked during the vulnerable period and which is increased by interventions that enhance susceptibility to fibrillation (*22–24*). Smith and Cohen proposed that alternans may be due to the summation of electrical activity of subpopulations of myocardial cells that generate action potentials only on alternate beats (*25*). Others have ascribed alternans to the action potential morphology of the individual myocardial cells (*15, 26*). Priori and co-workers observed that during coronary artery reperfusion 2:1 block of early afterdepolarization (EAD) conduction occurred simultaneously with the onset of T wave alternans in the intracavitary ECG (*27*). El-Sherif and coworkers reported summation of repolarization activity due to EADs in animals treated with the inotropic agent anthopleurin A (*28*).

From a biophysical perspective, T wave alternans may represent a prechaotic state because bifurcative behavior is the hallmark of chaos (*29–33*). Recent studies by Chialvo and others indicate that myocardial cells can exhibit chaotic dynamics (*29, 30*). To establish with certainty that T wave alternans represents prechaotic behavior requires demonstration of multupling just before fibrillation occurs. Although T wave multupling has been observed during infusion of high concentrations of norepinephrine (*31*), the progression to lethal arrhythmia remains to be demonstrated. This may prove elusive because higher order bifurcations represent extremely unstable, evanescent states.

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Recombinant Virus Vaccine–Induced SIV-Specific CD8$^+$ Cytotoxic T Lymphocytes

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Evidence indicates that cytotoxic T lymphocytes (CTLs) may be important in containing the spread of the human immunodeficiency virus (HIV) in the infected host. Although the use of recombinant viruses has been proposed as an approach to elicit protective immunity against HIV, the ability of recombinant viral constructs to elicit CD8$^+$ CTL responses in higher primates has never been demonstrated. A live recombinant virus, vaccinia–simian immunodeficiency virus of macaques (SIV_{mac}), was used to determine whether such a genetically restricted, T lymphocyte–mediated antiviral response could be generated in a primate. Vaccinia-SIV_{mac} vaccination elicited an SIV_{mac} Gag-specific, CD8$^+$ CTL response in rhesus monkeys. These CTLs recognized a peptide fragment that spans residues 171 to 195 of the Gag protein. The rhesus monkey major histocompatibility complex (MHC) class I gene product restricting this CTL response was defined. Both the vaccinated and SIV_{mac}-infected monkeys that shared this MHC class I gene product developed CTLs with the same Gag epitope specificity. These findings support the use of recombinant virus vaccines for the prevention of HIV infections in humans.

LIVE, ATTENUATED VIRUSES HAVE been used when possible in vaccination, as they elicit longer lasting immunity than that achieved by inactivated virus or protein immunization (*1*). However, in view of the propensity of HIV to undergo rapid mutations (*2*), such an approach for the prevention of acquired immunodeficiency syndrome (AIDS) does not seem feasible. The use of recombinant viruses may result in a level of immunity comparable to that attained in live viral infections (*3*). Thus, HIV genes encoding proteins crucial for eliciting immunity might be incorporated into an established, tolerated viral vector. Although recombinant viruses

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