Studies of the Equivalent Cardiac Generator Behavior of Isolated Turtle Hearts

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ABSTRACT
Equivalent cardiac generator components were determined for a series of excised turtle hearts immersed in Ringer's solution. Relatively large preparations were contained within a specially designed spherical chamber, and electric field potentials were derived from 20 evenly spaced electrodes on the chamber wall. A laboratory computer was used to acquire and store the 20 leads of signal data in digitized form in real time. An eccentric dipole was optimally fitted to the surface potentials for each 2 msec sample; the remaining voltages were used to determine a centric multipolar series through octapolar content. In addition to the purely quantitative parameters which were thus determined, sequential mapping of isopotential distribution over the spherical boundary gave valuable qualitative insights into the behavior of the equivalent generator throughout ventricular depolarization. This activity varied in complexity from predominantly dipolar to strongly nondipolar among different preparations. Peak quadripolar activity ranged from a low of 10 to a high of 60%, the corresponding figures for octapole content were 5–61%. The overall technique permits experimental exploration of several theoretical principles which have been advanced since 1954. Pilot studies on rabbit hearts indicate that the method will also be applicable to mammalian hearts.

KEY WORDS dipole quadripole octapole eccentric dipole equivalent cardiac generator isolated turtle hearts

A major goal of electrocardiographic research is to evaluate the generator properties of the heart as accurately as possible from the electrical field which surrounds it. Although still far from being fully achieved, this task may be simplified in purely experimental situations by supporting the beating heart in an artificial medium whose geometric configuration and physical characteristics are precisely known.

We have recently developed a technique of this kind, in which an isolated rabbit or turtle heart is contained within an accurately fabricated spherical chamber and electrocardiographic signals are recorded from numerous electrodes located on the inner surface of the chamber. The chamber is fairly small, having a diameter of 6.35 cm and is constructed in the form of two hemispherical portions which bolt together. Since the two halves are separable, relatively large hearts can be placed within the chamber. Its inner wall is studded with 20 uniformly distributed electrodes.

Methods and Materials

The structure of the isolated heart tank is illustrated in Figure 1. The two halves of the tank are cast from epoxy. Accuracy of the internal surface is ensured by the use of a precision-ground stainless steel sphere as a core in the fabrication process. Each electrode is equidistant from its three nearest neighbors, the angle of separation being $\tan^{-1} \sqrt{0.8}$.

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Illustration of the spherical chamber developed for studying the electrical fields generated by isolated beating turtle hearts. The two halves are constructed with exact centric symmetry except for an adjustable support structure which is built into the wall of one of the hemispheres. Chamber diameter is 6.35 cm. The inner surface is studded with 20 specially configured and evenly spaced sensing electrodes. Further description in text.
species also. The turtle hearts ranged in weight from 6.8 to 16.7 g, obtained from turtles weighing up to 4 kg.

Data Processing and Results

Single-Beat, Averaged, and Unipolar Signals.—The 20 preamplifiers were precisely calibrated at the beginning and end of each experiment with a very stable 10-Hz square wave standard. The QRS complex normally lasted from 200 to 300 msec. Data records consisted of blocks of digitized wave forms which were converted at a rate of 500 samples/sec for 400 msec. These records were long enough to include all of QRS, a short length of the ST segment, and a sizable length of base-line leader.

Examples of raw and processed signal information obtained from a single experiment are illustrated in Figures 2-4. Figure 2 is a plot of the 20 QRS complexes recorded and stored in digitized form for a single cardiac cycle. Since the plots are incremental, the appearance of noise in the illustration is a threshold phenomenon, first becoming perceptible at a level of 6 μv as referred to input. In this context, it is apparent that input noise is less than 6 μv in a number of the records, and not very great in any of the leads.

In dealing with preparations of this kind, it is of crucial importance that they remain physiologically stable over reasonable periods of time, and that sequences of successive wave forms exhibit a high grade of repeatability. The tape-stored digitized data are routinely tested for these attributes by computing a form of correlation coefficient which we have previously referred to as the "wave-form index" (WFI) (2). In essence, a wave form of appreciably good quality is selected as a standard of comparison from the early portion of a given sequence of beats. Following this choice, a least-squares determination is made of how much of the standard wave-form configuration is contained in all complexes. These calculations are carried out for all 20 leads, and the strings of successive WFI values are computer-plotted as "wave-form index profiles." The latter serve to depict the trend of configurational change and deterioration of the preparation.

In a typical experiment, there are no important beat-to-beat differences in the WFI profiles, with changes of generally less than 2% over a 5-minute period. In leads with relatively large peak-to-peak amplitudes, the rate of change tends to be considerably less than this figure. Having thus obtained assurance that the preparation is stable and...
repeatable, a sequence of 16 consecutive beats is selected and averaged over each of the leads. As illustrated in Figure 3, signal averaging of a 16-beat sequence suffices to reduce manifest random noise below the 6 µV level. In addition, the leading segment becomes quite straight and horizontal, thus permitting a base-line reference level to be selected with considerable confidence.

At this point, the following check is made on the overall precision of the technique, particularly of the relative accuracy with which the preamplifiers are calibrated. It will be recalled that the 20 bipolar leads are chained together to form a closed Kirchoff's loop. This being so, the sum of all areas such as those contained between the vertical lines in Figure 3 should be zero. Closure error is determined from the formula

$$CE = \frac{\sum_{i=1}^{20} \sum_{j=1}^{N} v_{ij}}{\sum_{i=1}^{20} \sum_{j=1}^{N} |v_{ij}|} \times 100\%,$$  \hspace{1cm} (1)$$

where $CE$ represents closure error, $v_{ij}$ is the potential in the $i$th lead at the $j$th instant of sampling, and $N$ is the number of samples in the given sequence. Closure errors of 0.5%, or slightly less, are obtained in a typical experiment.

The complexes are now reduced to unipolar form (Fig. 4) by referencing the potential of each electrode against a central terminal which represents the average potential of all 20 electrodes. As an example, this is accomplished in the case of electrode 1 by means of the following computation:

$$u_1 = (v_1 - v_2) + 0.95(v_2 - v_3) + 0.10(v_{19} - v_{20}) + 0.05(v_{20} - v_1),$$

$$+ 0.10(v_{19} - v_{20}) + 0.05(v_{20} - v_1),$$

where $u_1$ is the desired unipolar potential of electrode 1, $(v_1 - v_2)$ is the bipolar potential difference recorded between electrodes 1 and 2, etc. The terms in the preceding formulation can be gathered together as

$$u_1 = v_1 - \frac{1}{20} \sum_{i=1}^{20} v_i \hspace{1cm} (2a)$$

thus showing that the potential of electrode 1 has been referenced to the average of all 20 electrodes. A 5% loss of signal strength appears in the expression because the electrode itself is included in the computed central terminal. In experimental application, the computed unipolar values are scaled upward to account for
This illustration shows the 20-lead, averaged complexes of Figure 3 after they were referenced by numerical computation to a central terminal which represents the average voltage of all 20 electrodes. Time slew of approximately 55 μsec between successive channels is corrected by second-degree curve fitting and interpolation. These 20 unipolar leads are sorted into ten pairs of push-pull (odd symmetry) signals which just suffice to determine the three dipolar and seven octapolar components of the equivalent cardiac generator. The residual voltages form ten pairs of push-pull (even symmetry) signals, from which quadripolar content is determined by least-squares fitting.

To simplify the calculation of equivalent generator content, which will be described in the next section, pairs of unipolar potentials derived from diametrically opposite electrodes are sorted into their odd and even fractions.

**Centric Equivalent Generator Components.**

After data processing has advanced to the stage described above, the source of the surface potentials is resolved into a series of dipolar and multipolar generators (3, 4), mathematically expressed as the multipolar expansion

$$u_s = \frac{1}{4\pi\gamma} \sum_{n=1}^{\frac{n+1}{2}} \frac{2n+1}{n R^{n+1}} \left[ a_{n0} P_n^0 (\cos \theta) + \sum_{m=1}^{n} (a_{nm} \cos m\phi + b_{nm} \sin m\phi) P_n^m (\cos \theta) \right],$$

where $u_s$ is potential at the chamber surface, $\gamma$ is the specific conductivity of the turtle Ringer’s solution, $R$ is the inner radius of the spherical tank, $a_{nm}$ and $b_{nm}$ are the coefficients of the equivalent generator components, $\theta$ and $\phi$ are the elevation and azimuth, respectively.

Odd fraction is half the potential difference between a pair of diametrically opposite electrodes, even fraction is half the sum of their unipolar potentials.

Even fractions. When Eq. 3 is limited to odd degree (i.e., $n = 1, 3$), and the odd fraction of unipolar potentials is entered as $u_s$, a set of ten simultaneous linear equations is formulated, which just suffices for calculation of the 3 dipolar and 7 octapolar components of the equivalent generator. For $n = 2$, and entry of the even fraction of unipolar potentials as $u_s$, another set of ten equations is established, from which a least-squares solu-
Root mean square (rms) values of chamber surface potential during ventricular depolarization of two representative turtle heart preparations. Each of the two examples is illustrated by a family of curves (A and B). In each panel the uppermost curve (DATA) of the family depicts the rms value of QRS signal voltage; then, in descending position, the "data" curve is resolved into its centric dipolar (D), quadripolar (Q), octapolar (O), and residual (R) content. Due to mathematical orthogonality the data curve is the Pythagorean, rather than the algebraic, sum of the four component curves. The nature of the equivalent generator in examples A and B is discussed in greater detail in the text and illustrated further by the chamber surface iso-potential maps shown in Figures 6 and 7 for the instants of time I–IV.

Each panel represent, in order, resolution of total rms surface potential into its dipolar, quadripolar, octapolar, and hexadecapolar components.

The two examples were selected for illustration because of some rather striking differences in behavior. The curves on the left appear to be largely in phase since they all tend to rise rather than algebraic, sum. For example, the rms potential due to all quadripolar components is the square root of the sum of squares of all five $\tilde{a}_{2m}$ and $\tilde{b}_{2m}$.

Figure 5 depicts two examples in which chamber wall voltages have been resolved into centric dipole-multipole series. Each panel of the illustration shows a family of time-dependent curves. The uppermost of these curves represents the total rms value of electrocardiographic potential over the inner tank surface. The successively lower curves in each panel represent, in order, resolution of total rms surface potential into its dipolar, quadripolar, octapolar, and hexadecapolar components.

The usefulness of the equivalent generator coefficients is enhanced by converting them to the root mean square (rms) form (5, 6)
together, reach peak values at about the same time, and then subside simultaneously. In contrast, the right-hand family of curves differs particularly by showing delayed onset of the quadripolar (Q) contribution, with its rapid rise not occurring until about the peak of dipolar activity. The quadripolar function then goes on to reach a sizable peak during the phase of rapid dipolar falloff.

It is evident in both examples that an appreciable and quantifiable amount of octapolar moment is developed during ventricular depolarization, although it is not known at this stage of data reduction what proportion is due simply to generator eccentricity. Example B shows the equivalent generator to be predominately dipolar through the first one-third of QRS interval, with transition to strongly quadripolar behavior by the end of the second one-third of the interval. In contrast, the phase-coherent nature of the function curves in example A suggests that the equivalent generator in this case behaves predominantly as an eccentric dipolar source. These two apparent extremes of behavior will be examined in greater detail in the following sections.

Isopotential Mapping.—The foregoing mathematical formulations indicate how 24 dipolar and multipolar equivalent generator components are extracted from each temporal set of 20 data points. It is believed that the first 15 of these, embracing the dipolar through the octapolar series, possess genuine quantitative value. The remaining 9 coefficients give the appearance of quantitative hexadecapolar components, but serve mainly as “trimmers” which permit the 20 data points to be reconstructed exactly from Eq. 3. Fortunately, the hexadecapolar content is generally found to be quite small; otherwise its presence could seriously interfere with evaluation of quadripolar content.

Once the coefficients $a_{nm}$ and $b_{nm}$ of Eq. 3 have been fully determined, it is largely a matter of computer “housekeeping” to interpolate electrocardiographic voltages between the 20 electrodes on the surface of the tank.
Chamber surface isopotential maps corresponding to example B of Figure 5. Same coding of voltage positivity and negativity as Figure 6. Early in depolarization (instant I) the generator is relatively weak and manifestly dipolar. By instant II the isopotentials about the surface maximum have begun to flatten. Panel III indicates that the flattening has proceeded to actual pinching off with the formation of two surface maxima (one behind the plane of projection) and one minimum. This configuration can be construed as representing a predominantly dual dipolar generator, which is manifested in Figure 5B by its strong quadripolar effect. Panel IV shows isopotential distribution during subsidence of generator activity.

and generate displays of electrical field in the form of surface isopotential maps. These maps can be drawn out on an incremental plotter or, alternatively, they can be shown as cathode-ray tube displays. In the latter form, it has proved feasible to photograph numerous successive frames and thus generate animated films of the electrical fields which are associated with ventricular depolarization.

Such films have been made of the "dipolar" and "quadripolar" examples which are illustrated in A and B, respectively, of Figure 5. The example A sequence, illustrated in Figure 6, shows only one surface maximum and minimum of potential throughout QRS, as is characteristic of a persistently dipolar source. Further inspection of the maps also indicates that the source is changing in strength, orientation, and probably location, throughout the cycle. Four frames from the quadripolar case are shown in Figure 7. Frames III and IV indicate the development of two surface maxima of potential, as might well be expected in strongly quadripolar behavior caused by a predominantly dual dipolar source.

**Eccentric Dipole Location.—** Using the coefficients \( a_{nm} \) and \( b_{nm} \) of dipolar and quadripolar components, a set of five simultaneous linear equations may be formulated which involve \( x \), \( y \), and \( z \), the coordinates of dipole location, as unknowns (5, 6). For use with the tank, however, a gradient method of determining dipole position has been developed which is intended to minimize those errors arising from highly eccentric dipole location.

The procedure is an iterative one, and uses the results of the earlier method (5, 6) as initial values.

Briefly, the iterative computations are based on the following principles. Designating the lead field potential (7, 8) associated with a given electrode as \( \Phi \), and the components of a dipole generator in the chamber as \( M_x, M_y, M_z \),
FIGURE 8

Representation of the equivalent cardiac generator as a dipolar singularity whose location moves throughout ventricular depolarization. The upper row of illustrations shows three different views of a given turtle heart preparation. Shown immediately below each view are the corresponding projections of the conventional dipole moment loop, including projection of \( x, y, \) and \( z \) reference axes. Superimposed on each anatomical figure is shown the pathway which the equivalent dipole follows during genesis of QRS forces.

and \( M_z \), the voltage \( V \) produced at the electrode is

\[
V = \frac{\partial \Phi}{\partial x} M_x + \frac{\partial \Phi}{\partial y} M_y + \frac{\partial \Phi}{\partial z} M_z.
\]

The difference between the potential actually recorded at this particular electrode and the potential produced by the hypothetical dipole is determined in approximate form, as shown below, by applying the chain rule to the preceding expression for \( V \), giving

\[
V_{obs} - V = \Delta V = \frac{\partial V}{\partial x} \Delta x + \frac{\partial V}{\partial y} \Delta y + \frac{\partial V}{\partial z} \Delta z + \frac{\partial \Phi}{\partial x} \Delta M_x + \frac{\partial \Phi}{\partial y} \Delta M_y + \frac{\partial \Phi}{\partial z} \Delta M_z.
\]

The last three terms stand as shown. The first three terms of the chain require further expansion, as, for example

\[
\frac{\partial V}{\partial x} = \frac{\partial^2 \Phi}{\partial x^2} M_x + \frac{\partial^2 \Phi}{\partial x \partial y} M_y + \frac{\partial^2 \Phi}{\partial x \partial z} M_z.
\]

The deviations of electrode voltage, \( V_{obs} - V \), can then be expressed as a function of six incremental variables, \( \Delta x, \Delta y, \Delta z, \Delta M_x, \Delta M_y, \Delta M_z \). Due to the known geometry and other volume conductor properties of the spherical tank the first- and second-order partial derivatives of lead field potential can be evaluated. Because of the 20 electrodes, there are 20 equations of the above form from which a least-squares fit of the six incremental variables can be determined. Beginning with the aforementioned initial values, the adjustments of location and electrical moment are calculated and applied iteratively until maximum improvement is attained.

Results obtained from application of the method to a complete temporal sequence of data can be displayed in the form of two kinds of vector loop. The first of these is the well-known loop of dipole moment, which defines the termini of successive instantaneous heart vectors. The second kind, which is decidedly less well known, defines the location of the mobile equivalent dipole generator. An example of these two complementary modes of equivalent generator representation is shown in Figure 8. The electrical moment loops are depicted in three different projections. Immediately above each loop, the locus of dipole position is shown in the same projection.
TABLE 1
Source of Electric Potentials on Chamber Surface Based on Resolution of Equivalent Cardiac Generator into Eccentric Dipole and Centric Dipole-Multipole Series

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See text for description and discussion.
*Area is given in μv-sec, and peak in μv.
†Eccentric dipole could not be computed for heart 15 because of strongly nondipolar generator characteristics.

overlaid on the corresponding view of the heart. Although data are not available from which the correctness of loop construction might be verified, the derived positional information appears plausible to the extent that the dipole locus remains within the confines of the cardiac region.

Optimized fitting of an eccentric dipole was applied to all 14 isolated heart preparations, with the results summarized in Table 1.
Column 1 of the table presents the time-integrated (area) and peak values of the rms voltages obtained over the 20 electrode sites. An eccentric dipole was determined for each 2-msec sampling interval, and the contributions of these to total surface data are shown in relative units in column 2. The actual contribution of the eccentric dipoles to surface electric potential was subtracted from the original data, and the remainders were used to determine a centric multipolar series as indicated in columns 3–5 of the table. Column 6 expresses the amount of potential which was not accounted for by the two kinds of equivalent generator computations.

Because the principle of Pythagorean summation (or, more accurately stated, subtraction) is involved in these computations, considerable surface potential remains even after the contributions of, say, a 95% eccentric dipole source are removed. As shown in the area rows of the table, varying amounts of centric generator contribution were obtained from these remainders. Not surprisingly, centric dipole strength was quite small in all examples. Quadrupolar contributions ranged from a low area value of 6% in heart 6 to a high of 30% in heart 18, with peak values of 10% and 60%, respectively. Heart 20, the highly nondipolar characteristics of which are illustrated in Figures 5B and 7, is confirmed by the tabulated data as another strongly quadrupolar example. Heart 16, which is presented in Figures 5A and 6 as exemplifying single mobile dipole behavior, shows area and peak quadrupolar values of 14% and 10%, respectively. This is quite a lot less than the Q curve of Figure 5A indicates; the difference is due to the separation of purely intrinsic quadrupolar components from those owing solely to eccentric dipole location.

Column 5 of the table shows that significant amounts of octupolar content may be present. The values range from a low of 6% overall and 5% peak for heart 6 to a high of 20% overall and 61% peak for heart 19.

Discussion

Although electrocardiographic registration from electrolyte-filled tanks containing isolated hearts is not new (9–11), we have attempted in this study to combine a number of individually meritorious technical features into an especially accurate experimental system for the acquisition and subsequent computer processing of multiple electrical signals. The individual features include (1) relatively small chamber size, (2) efficient distribution of electrodes over the chamber wall, (3) point-sensor characteristics of electrodes, (4) precise calibration methods, (5) virtually simultaneous registration of multiple leads, and (6) quality check of signal wave forms.

Due to the relatively small size of the chamber, peak unipolar potentials of 350 µV or more were not uncommonly encountered. This bespeaks a favorable signal-to-noise ratio when compared to 2.8 rms µV of preamplifier noise. Signal quality was further enhanced by 16-beat averaging of wave forms which had been carefully checked for repetitive configuration. Of even greater importance, small chamber size contributes to the successful extraction of multipolar generator content by avoiding excessive dissipation of quadripolar and octupolar components. In a larger chamber these would be attenuated according to the third and fourth power of radius, respectively (Eq. 3).

Figure 2 illustrates that rather good signal quality can be achieved with single-beat recording. However, an essential feature of our data reduction method is numerical referencing of individual electrode potentials to the average of all 20 (so-called central terminal). This computational maneuver requires precise determination of bipolar lead base lines which, as is implicit in a comparison of Figures 2 and 3, can be achieved by 16-beat averaging. It seems likely that achievement of the necessary precision may be the major contribution of signal averaging to the data processing.

In a meticulous study of an isolated turtle heart, Taccardi (12) demonstrated that predominantly dipolar electrical fields were generated during about the first two-fifths of...
ventricular invasion. Later in ventricular activation, the electrical field maps became more intricate and resembled those which would be produced by a pair of normally oriented dipoles located toward the right and left basilar portions of the ventricles. A few of our preparations (one illustrated in Figures 5B and 7) similarly showed initial dipolar behavior with eventual metamorphosis into a manifestly dual dipolar generator. In other preparations, as illustrated, for example, in Figures 5A and 6, generator behavior was predominantly dipolar throughout all of ventricular activation.

Resolution of the electromotive forces of the heart into a single moving dipolar source has been an attractive research goal which was first suggested in 1954 by Gabor and Nelson (13), and has recently been achieved with a human subject (14). Theoretical formulations exist from which the location-dependent fraction of quadripolar content can be translated into terms of equivalent dipole location (5, 6), leaving a residuum of invariant quadripolar information. In the advanced form of solution developed for this study, truncation error arising from exaggerated degrees of dipole eccentricity is greatly minimized through introduction of an appropriate iterative numerical method.

Attempts at eccentric dipole fitting are foredoomed to failure unless the generator is sufficiently dipolar in nature, although what constitutes “sufficiency” is far from clear. An extreme “insufficient” case is the hypothetical example of a generator which consists of two equal and oppositely directed dipoles. There is no net dipole moment in this situation, and an eccentric dipole generator cannot be determined for it. On the other hand, it may be emphasized that dipole fitting is a very useful procedure when dipolar content predominates in the voltage sources.

The physical and physiological significance of quadripolar content may likewise be obscure. Eccentric location of a dipole gives rise to manifest quadripolar moment, which is expressed mathematically (5, 6) as weighted sums of first-order locational product moments of the dipolar components. Therefore this kind of quadripolar content disappears when the origin of the reference system is translated to the dipole site. A pair of separated dipoles also gives rise to quadripolar moment which does not wholly disappear despite optimized choice of reference system origin. Other kinds of source-sink distributions, such as electromotive surfaces, produce quadripolar moment. The magnitudes of such moment is location-dependent and can, in principle, be reduced to some minimum value by appropriate translation of the reference system origin.

The significance of the octapolar content which was determined for the series of isolated turtle heart preparations (column 5, Table 1) is also not entirely clear at this time. If ventricular depolarization in this species were to proceed in the form of one or more electromotive surfaces, as appears to be the case in the canine and human heart, it is theoretically inevitable that significant octapolar activity would be associated with the process. Further extrapolation from theoretical considerations (6, 15, 16) suggests that octapolar content might be quite large with respect to quadripolar activity in the case of a single electromotive surface, whereas these two multipolar fractions would tend to parallel each other if depolarization were to consist of two or more electromotive surfaces, or were to assume some other nonsimple form. Inspection of Table 1 indicates that there is a crude correspondence between the magnitudes of quadripolar and octapolar effects, suggesting that a single electromotive surface would be too simple a representation of ventricular depolarization.

The chamber has been designed to promote effective application of many of the theoretical advances which have emerged over the past 17 years. The spherical shape of the chamber is, of itself, exceptionally well suited to the surface spherical harmonics form of multipole expression which is obtained when radius is constant. Once technical difficulties were overcome, it was gratifying to note that the on-line processing of multichannel signal information could be handled very satisfactorily by the laboratory-oriented computer.
Extrapolating from our current level of experience, we anticipate the later development of systems in which about half again as many channels of information will be handled on-line. Apart from these purely technical projections, the existing 20-channel system described in this report already produces large volumes of information with unprecedented accuracy, and thereby provides valuable new insights into equivalent generator behavior.

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