more damaged if the capsule was removed. Although current passage through a recording electrode can introduce errors in the measurement of resistance, care was taken to keep the bridge in balance. This is not particularly difficult with electrodes having resistances in the range of 15 to 30 megohms and with maximum currents of the order of 10^{-8} amp. Another danger is that the electrode resistance changes during the current passage by depleting ions from the tip. Any change of electrode resistance is easily detected as a change in bridge balance between the onset and end of the current pulse (usually about 100-msec duration). Results were discarded when such changes in balance occurred.

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12. The R_m can also be calculated from a measurement of total surface area and input resistance. For a 150-μm cell, surface area, assuming no indentations of the surface membrane and assuming the cell to be a sphere, would be 6.9×10^{-4} cm^2. For a sphere with an input resistance of 10^7 ohms there would be a limiting R_m of 6900 ohm cm^2. Some molluscan and many other invertebrate neurons have membrane indentations that increase the total surface area by up to 7.5-fold [M. Mirolli and S. R. Talbott, *J. Physiol. London* **227**, 19 (1972); T. H. Bullock and G. A. Horridge, *Structure and Function in the Nervous Systems of Invertebrates* (Freeman, San Francisco, 1965)]. Information as to the extent of indentations of the squid soma membrane is not available. In order to have agreement between the two calculations there would have to be a four-fold increase in surface membrane area over that of a single sphere.
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14. These experiments were done at the Marine Biological Laboratory, Woods Hole, Massachusetts. I thank K. S. Cole and W. J. Adelman, Jr., for the use of their facilities and for discussions, and A. F. Bak for assistance.

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Electrocardiogram Monitoring: Computerized Detection of Ventricular Changes Induced by Drugs

Abstract. *Cardiac arrhythmia was induced in mice by the injection of ouabain, lidocaine, and diphenylhydantoin. Temporal spreading of ventricular electrocardiographic complexes is shown consistently to follow injection of a cardioactive drug and to precede rhythm disturbances. A computerized monitoring technique based on temporal spreading and waveform distortion should find wide clinical and experimental application.*

Cardiac arrhythmias resulting from therapeutically administered digitalis, quinidine, procainamide, and lidocaine or from abnormal concentrations of blood electrolytes pose serious health problems (*1*). Associated premature ventricular contractions (PVC) and ventricular fibrillation which arise in these situations appear secondary to cardiac conduction impairments (*2*). Most advanced monitoring programs rely upon the detection and classification of rhythm disturbances obtained by a continuous, real time analysis of the electrocardiogram (ECG) (*3*). Such programs, however, are insensitive to gradual ECG changes which may precede critical rhythm disturbances. Detection should include indications of pending arrhythmias so treatments might be offered or modified to avoid patient exposure to critical cardiac conditions. Our objective was to determine whether such detection criteria can be physiologically and technically satisfied.

An initial assumption was that decreases in propagation velocity along the Purkinje network would cause delays in muscle activity proportional to distance. Thus, if a cardiac treatment operated homogeneously on Purkinje cells, simple spreading or time scaling of ventricular ECG complexes is expected. Selected effects might be expected to produce distortions in addition to time spreading since cardiac muscle masses would be activated in abnormal sequences. Accordingly, the computer was programmed to detect the time spread of QRS waveforms from samples of ECG's obtained both before and after drug treatments that produced cardiac arrhythmias. In addition, waveform correlation methods were designed to measure residual ventricular alterations not due to simple time spreading.

Table 1. Summary of results obtained with lower doses of ouabain (Ouab), lidocaine (Lido), and diphenylhydantoin (Dph). Latency shows elapsed time between treatment and first β alterations. Minimum β and qualitative alterations in the ECG are noted, and both occurred near the end of each recording session. The ρ_{max} was more than .95 during periods prior to arrhythmia.

Dose (mg/kg)	Latency (min)	Minimum β	Rhythm disturbances	Death
0.9 (Ouab)	45	0.82	None	No
3.4 (Ouab)	23	.75	PVC's	Died within 24 hours
4.0 (Ouab)	8	.62	PVC's and tachycardia	No
10.0 (Ouab)	4	.50	PVC's and fibrillation	Died within 2 hours
10.0 (Lido)	1	.87	None	No
10.0 (Dph)	60	.92	None	No
30.0 (Dph)	23	.87	PVC's	No

Twenty adult mice (weighing 28 to 32 g) were each anesthetized briefly with chloroform, and transthoracic electrodes, consisting of 9-mm stainless steel wound clips attached superficially to the epidermis, were implanted bilaterally. Flexible spectrastrip cable carried ECG signals from the mice to preamplifiers. During recording sessions, half the mice were restrained in a Plexiglas neck and leg harness and the other mice were lightly tranquilized with chlorpromazine (1 mg per kilogram of body weight). In either case a 30-minute period of adaptation was allowed immediately following implantation of electrodes and the ECG was continually monitored for several hours in all mice.

The ECG signals were amplified and recorded. Analysis began with filtering (1.6- to 250-hertz band pass, 60-hertz notch), synchronizing (narrow bandpass filter at 105 hertz followed by threshold detection), and digitizing (1000 samples per second) of the recorded signals. The synchronization pulse marked the QRS complex for digital sampling. Initially, 20 consecutive ECG beats were sampled and averaged to provide a reference waveform, $S_R(t)$.

Subsequent 20-beat samples were similarly averaged for continuous comparisons with the reference. Temporal position and time scale variables were searched iteratively to maximize the cross-correlation coefficient, ρ, between the reference waveform and each new averaged waveform. The search of the temporal position variable eliminated false discrepancies between reference and compared waveforms due to synchronization variations. The maximized ρ is thus described in the following way

$$\rho_{max} = \max_{\{\beta,\tau\}} \left\{ \frac{\int_0^T S_R(\beta(t-\tau))\, S_c(t)\, dt}{\left[\int_0^T S_R^2(\beta(t-\tau))\, dt \cdot \int_0^T S_c^2(t)\, dt \right]^{1/2}} \right\}$$

where $-1 \leq \rho_{max} \leq 1$; $S_R(t)$ is the reference; $S_c(t)$, the current 20-beat average; β, the time scale variable (spread factor); τ, the temporal position variable; and T, the correlation interval (40 msec centered by synchronization pulse). Amplitude variations were omitted (normalization via integral square terms in denominator), since such variations were most often related to respiration, movement, and slight electrode displacement. The actual implementation was digital and described by the following equation (where ΔT is time between samples)

$$\rho_{max} = \max_{\{\beta,\tau\}} \left\{ \frac{\sum_{i=1}^{N} S_R(\beta(i \cdot \Delta T - \tau))\, S_c(i \cdot \Delta T)}{\left[\sum_{i=1}^{N} S_R^2(\beta(i \cdot \Delta T - \tau)) \cdot \sum_{i=1}^{N} S_c^2(i \cdot \Delta T)\right]^{1/2}} \right\}$$

The reference waveform, S_R, is obtained by storing the first 12 coefficients in a Fourier series expansion of a 20-beat average waveform taken at the start of monitoring. The search over β and τ is accomplished by time shifting and time scaling the sinusoidal terms in the series as S_R is reconstructed and cross correlated with S_c. The maximization is accomplished by a quantization and search over β and τ starting at those values which maximized the previous 20-beat average, S_c.

The analysis of records obtained from untreated or saline-treated control mice showed that the QRS complex was an extremely stable waveform despite large variations in other ECG indices, such as heart rate. The spread factor, β, varied over less than a 5 percent range around a mean value of unity, and ρ_{max} remained more than .95 while heart rates varied from 120 to 420 beats per minute (Fig. 1, A and C). The present characterization thus appeared to produce a very stable baseline from which to assess the effects of several potent cardioactive drugs.

Lethal doses of ouabain (10 mg/kg) were employed in several mice to establish definite cardiac rhythm disturbances. Both the QRS complexes and heart rate were monitored. Rapid transitions in heart rate and time spread factor occur together with smooth, progressive decreases in the latter (indicating spreading) after ouabain injections (see Fig. 1, B and D). The maximized correlation coefficient, ρ_{max}, remained above .95 until 10 minutes before fibrillation and death. Thus, ouabain produced initial waveform changes that consisted of pure time spreading. Although heart rate decreased rapidly (latency $\approx$ 1 minute) from 400 to 250 (20-beat averages), the response was probably related to indirect, vagal effects of the cardiac glycosides (*4*) while time spreading was indicative of direct effects in the ventricles. In most cases a nearly pure time spreading of the ventricular depolarization complexes preceded the development of PVC's fibrillation or other aberrant activity (*5*).

Although the monitored ventricular indices were sensitive (detection of greater than $\pm$ 3 percent changes) and reliable for lethal drug treatments, it seemed possible that very mild treatments might escape detection by a monitor already shown insensitive to many variations in the normal cardiac cycle. Low doses of lidocaine, diphenylhydantoin, and ouabain were all used to test the sensitivity of the detection system. Regardless of drug or dose, the monitoring system detected drug treatments, and the saline control treatments showed that the effects were not due to injections alone.

Table 1 summarizes the results of these experiments. Steplike time spreading and prolonged plateaus of little or no spreading were often present in response to ouabain. The latency between injection and first observable ECG effect, as well as the minimum time-spread factor obtained in 4 hours of monitoring, appear dose dependent. Lidocaine hydrochloride doses (10 to 15 mg/kg) resulted in nearly immediate time spreading with latencies generally less than 1 minute. Complete transitions in the β monitor were more gradual, having a time constant of approximately 4 minutes at such levels of lidocaine hydrochloride. Changes in heart rate were either inconsistent or did not appear with such treatments. Treatment with diphenylhydantoin sodium produced time spreading with latencies similar to those obtained with ouabain. In all cases, a nearly pure time spreading of ventricular depolarization complexes followed drug injection and preceded the onset of arrhythmia. The minimum β was always obtained at or near the end of monitoring.

The results reported here indicate that ventricular depolarization events are frequently dissociated from factors that control cardiac events. The sensitivity of ventricular events to cardioactive drugs and insensitivity to normal

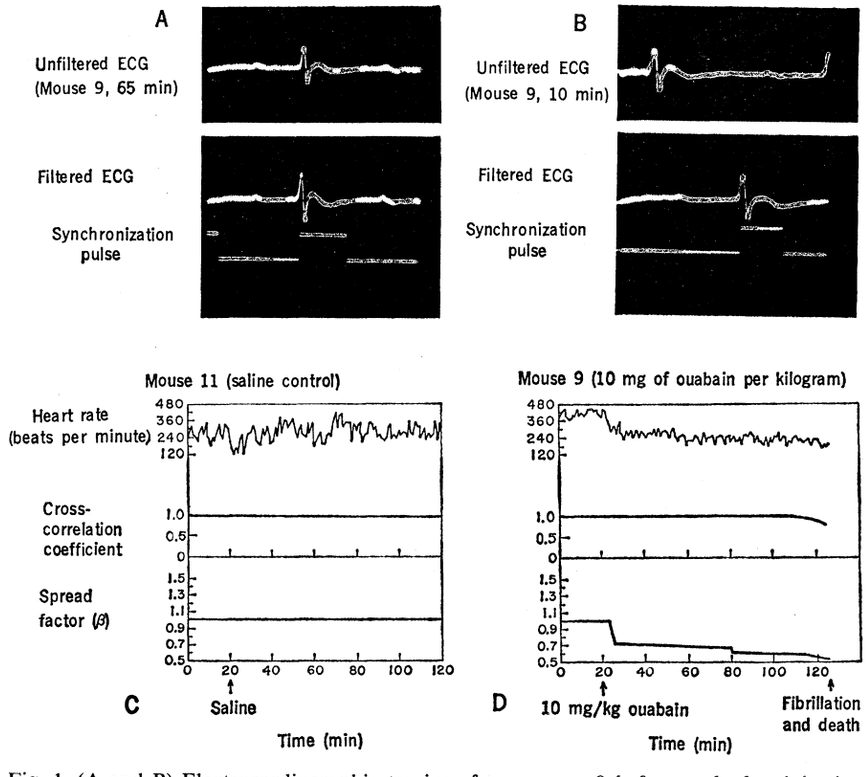

Fig. 1. (A and B) Electrocardiographic tracings from mouse 9 before and after injection of ouabain, respectively. Duration of synchronization pulse is 50 msec. (C and D) Heart rate, time spread, and correlation coefficient monitoring under injections of saline and lethal ouabain, respectively.

variations in heart rate make these events prime candidates for clinical monitoring. Since the detection scheme employed here depended on temporal spread factors, it is tempting to believe that small, subtle alterations in ventricular physiology might be detected from real time ECG analyses. Such detection should find exceptionally wide clinical and experimental usefulness. Finally, the concept of temporal spread might also prove useful for the analysis of other portions of the ECG.

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5. In some studies in which very high doses (20 to 30 mg/kg) were used, complete heart block occurred within 5 minutes of ouabain injection. Prior treatment with atropine eliminated vagal influences, and spreading responses were revealed, as discussed above, with the highest doses used (30 mg/kg).
6. Supported, in part, by Colorado Heart Association grant V71-72 and HEW grant 16-P-56815.
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Serotonin: Radioimmunoassay

Abstract. *The development of a radioimmunoassay for serotonin is described. Serotonin is made antigenic by coupling it to bovine serum albumin. Thrombocyte-free plasma instead of serum from the immunized animals is used for radioimmunoassay. Less than 1 ng serotonin can be measured by this procedure.*

The biogenic amine serotonin has been implicated as having a physiological role and has been suggested as being involved in many human diseases (*1*). An association has also been made between serotonin and emotional stability (*2*). To evaluate the role of serotonin it is critical to have a method that can measure the amine with great sensitivity and specificity in various biological fluids. Radioimmunoassay offers such a potential.

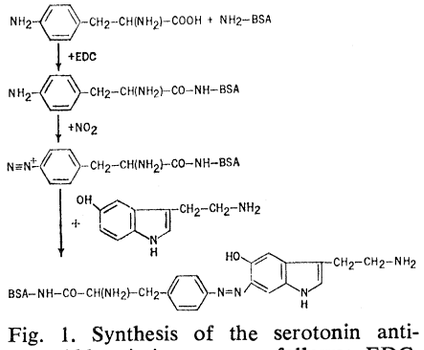

Fig. 1. Synthesis of the serotonin antigen. Abbreviations are as follows: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; BSA, bovine serum albumin.

Antibodies specific for serotonin have been described by others (*3*). However, these antibodies seem to have had generally low titers, and they have never been used for a radioimmunoassay. A number of methods for the determination of serotonin in biological fluids and tissues have been described (*4*). In this report we describe the production of antibodies against serotonin in rabbits and their use in a specific and sensitive radioimmunoassay.

To 50 mg of DL-*p*-aminophenylalanine, dissolved in 5 ml of distilled water, 50 mg of bovine serum albumin (BSA) and 50 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (*5*) were added. The mixture was incubated at room temperature overnight and then dialyzed for 5 days against distilled water with three changes (2 liters each) per day. Then the *p*H was adjusted to 1.5 with 1*N* HCl, and the following procedure was performed at 0° to 4°C. One hundred milligrams of $NaNO_2$, dissolved in 1 ml of distilled water, was added slowly dropwise, followed by 50 mg of ammonium sulfamate in 1 ml of distilled water. The diazotized protein solution was then added dropwise to 100 mg of serotonin creatinine sulfate, dissolved in 10 ml of 0.1*M* borate buffer, *p*H 9.0, with constant stirring. The *p*H was maintained above 8.0 by the addition of borate buffer. A dark red color developed almost immediately. The preparation was stirred overnight in the dark at 4°C and then dialyzed exhaustively against distilled water. For determination of the amount of serotonin coupled to the protein carrier 1 μc of [^{3}H]serotonin (*6*) was added with unlabeled serotonin, and the above procedure was followed. By measuring the radioactivity of the dialyzates and the antigen solution, it was calculated that 5.7 percent of the serotonin had been coupled, corresponding to about 20 moles of serotonin per mole of BSA (molecular weight, 70,000). Figure 1 illustrates the method of synthesis and the presumed structure of the immunogen.

Rabbits were immunized with 150 μg of the immunogen which had been dissolved in 0.5 ml of phosphate-buffered saline, *p*H 7.4, and emulsified with an equal volume of complete Freund's adjuvant; 0.25 ml was injected into each foot pad. Booster injections were given weekly for 2 weeks, and thereafter every 6 to 8 weeks. Bleedings were taken from the central ear artery, the first one as a control before immunization, and then 1 to 2 weeks after booster injections. In a few cases, the blood was allowed to clot at room temperature for 1 hour and at 4°C overnight, and then the serum was separated by centrifugation at 10,000 rev/min for 30 minutes. Most bleedings, however, were taken into 250 units of

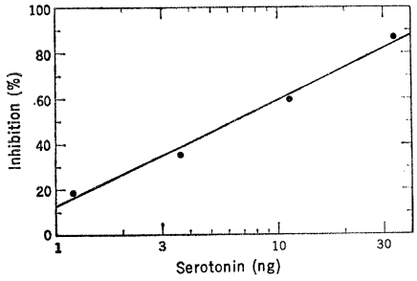

Fig. 2. Inhibition by nonradioactive serotonin of binding of [^{3}H]serotonin by antibody to a serotonin–bovine serum albumin complex (final dilution 1 : 50). The [^{3}H]-serotonin content of the assay mixture was approximately 2000 count/min. In the absence of nonradioactive serotonin, the antibody can bind 50 percent of the radioactivity.