Effects of Diphenylhydantoin on Excitability and Automaticity in the Canine Heart

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ABSTRACT

Studies were performed on the dog heart in situ to determine the effects of diphenylhydantoin (DPH) on excitability and automaticity. DPH decreased automaticity in the His-Purkinje system, evidenced by an increase in the ventricular escape time both during vagal stimulation in intact dogs and after ventricular overdrive in dogs with heart block. The spontaneous ventricular rate of dogs with heart block was unaffected by doses up to 20 mg/kg. DPH had no significant effect on either atrial or ventricular diastolic threshold when this variable was tested with bipolar stimuli or with stigmatic anodal or cathodal stimuli. The effective refractory period of atrial and particularly of ventricular muscle was shortened by DPH (10 mg/kg); a leftward shift in the strength-interval curve occurred consistently. Multiple response threshold and fibrillation thresholds were elevated by DPH in both the ventricle and atrium (20 of 24 experiments). In anesthetized dogs, intraventricular conduction velocity increased minimally after DPH administration. DPH increased transmembrane threshold voltage and reduced the current required to stimulate isolated Purkinje fibers. The commercial solvent used clinically as a diluent for DPH was found to increase diastolic threshold and prolong the effective refractory period.

ADDITIONAL KEY WORDS
fibrillation threshold
intraventricular conduction
strength-interval relationship
transmembrane threshold voltage

Antiarrhythmic drugs are thought to act by exerting effects on cardiac excitability, automaticity and conduction (1). The effects of diphenylhydantoin (DPH) on these variables remain controversial despite intensive study in the laboratory (2-8) and wide clinical use as an antiarrhythmic drug (9-18). In conflicting clinical reports, some groups report DPH to have effects similar to quinidine while others point out remarkable differences (11, 12, 15-18).

Laboratory studies designed to delineate the cardiac action of DPH have also led to conflicting results. Gupta et al. (19) suggested...
that DPH elevated diastolic threshold in ischemic ventricular muscle without altering the excitability of the normal myocardium. Rosati et al. (8), however, reported that this drug increased diastolic threshold of canine atrium in situ and ventricle in situ in a manner comparable to the changes seen with quinidine and found small, inconsistent changes in the effective refractory period. Studies of the effects of DPH on isolated canine Purkinje fibers (7) showed a shortening of the action potential and effective refractory period and a slight decrease in current requirement for stimulation.

DPH has also been shown to decrease automaticity in isolated canine Purkinje fibers (7) and to decrease ouabain augmented automaticity in the dog heart in situ (20). However, the effects of DPH alone on automaticity in the dog heart in situ have not been investigated systematically.

The present study was performed to further examine the effects of DPH on automaticity, excitability and conduction in the dog heart in situ and on threshold voltage in isolated canine Purkinje fibers. In the course of these studies it was found that the commercial diluent (40% propylene glycol, 10% alcohol, pH 12) in which DPH is dissolved for clinical use had significant effects on excitability when administered alone.

**Methods**

**EXCITABILITY**

Healthy mongrel dogs of either sex, weighing 12 to 20 kg, were anesthetized by pentobarbital sodium (25 to 30 mg/kg iv). Ventilation was maintained through an endotracheal tube with a Harvard respiratory pump. A right thoracotomy was performed and the pericardium opened, and acrylic plaques, each bearing five silver electrodes, were sutured to the right atrial and right ventricular epicardium. The electrodes were circular with diameters of 1 to 1.2 mm; the interelectrode distances on a plaque were 3 to 4 mm. A 24 by 5 mm rectangular electrode was sutured to the atrial or ventricular epicardium at a distance from the other stimulating and recording electrodes. This electrode served as an indifferent electrode so that stimulation at this site could be readily detected. In some experiments the sinus node was crushed to permit study at slower heart rates. The pericardial and thoracic incisions were then closed. The cervical vagus nerves were isolated for experiments in which vagal stimulation or section was employed. The femoral artery and vein were cannulated with polyethylene tubing and arterial pressure measured with a strain gauge pressure transducer (Statham p23D).

The surface electrocardiogram was recorded from subcutaneous steel electrodes. Temperature was continuously measured by a thermister probe placed in the retrocardiac esophagus (telemeter, Yellow Springs Instruments Co.) and temperature held constant between 37° and 39°C by a heating pad. Oxygen and carbon dioxide tensions and pH were estimated on arterial blood samples drawn before and during the measurement of excitability; the Po2 was 87 ± 2.5 (mean ± se), Pco2 was 36 ± 1.9 and pH was 7.35 ± 0.02 in these experiments.

The basic stimulating and recording arrangement is diagrammatically illustrated in Figure 1. The heart was driven at constant selected rates through one pair of electrodes with a rectangular pulse (S1), 5 msec in duration and twice threshold in amplitude. Test stimuli (S2) were delivered every sixth to tenth drive cycle at any desired interval after S1. The duration of S2 was varied according to the nature of the experiment. When measuring the multiple response threshold or fibrillation threshold of atrium or ventricle, a 180 volt battery was used as a voltage source. Pulses from 50 µa to those in excess of 30 ma could be obtained.

The interval between S1 and S2 was measured with a calibrated electronic digital counter (Hewlett-Packard, Model 523 DR).

S2 was measured by simultaneous display of its current (the voltage drop across a precision 1 Kohm resistor in series with the punctate electrode) and voltage on a calibrated storage oscilloscope (Tektronix, type 564). This allowed accurate (± 3% of full scale) measurement of these variables and a constant check on the impedance of the system.

Bipolar electrograms from various sites on the atria and ventricles, the surface electrocardiogram, and femoral arterial blood pressure were displayed and recorded on an eight-trace, switched-beam oscilloscope (Electronics for Medicine, Model DR 8). The testing procedure involved in measuring the strength-interval relationship, the latency between early S2's and their responses, the multiple response threshold and fibrillation thresholds were in general those used by Brooks, et al. (21). Significant departures...
EFFECTS OF DPH ON EXCITABILITY AND AUTOMATICITY

Block diagram of arrangement for stimulating and recording. Waveform generator (WG, A), acted as a clock for the system. Pulse generators (PG, A and B) could be connected through a junction box and isolation units to either the atrium or ventricle. During studies of atrial excitability, PG-B was not used and the test stimulus (S₂) was delivered to the atrium every sixth to tenth cycle from PG-C (electrode arrangement for atrial S₂ not shown). When ventricular excitability was being studied, PG-A paced the atrium and PG-B, the ventricle, to minimize interference from asynchronous atrial rhythms; S₂ was delivered by PG-C between a small punctuate electrode and a large indifferent electrode.

The time interval between S₁ and S₂ of the test cycles was measured and digitally displayed by the electronic counter. S₂ current and voltage amplitude was measured simultaneously on a dual beam oscilloscope. The atrial and ventricular electrograms, electrocardiogram and arterial pressure were monitored and recorded on the oscilloscopic recorder.

from their techniques are indicated in the results.

Preliminary experiments showed that intravenous doses of 5 mg/kg produced plasma DPH concentrations which fell below 10 μg/ml within 10 to 15 minutes after doses of 10 mg/kg, the plasma concentration remained above 10 μg/ml for at least 20 minutes. The larger dose allowed entire strength-interval or strength duration curves to be obtained while plasma concentrations were in a range comparable to the antiarrhythmic concentration of DPH in man (17). In some experiments, DPH (5 or 10 mg/kg body weight), dissolved in either saline or commercial diluent, was injected intravenously over a 3-minute period, and the variables under study were repeatedly measured to define the time course of DPH effect. In other experiments, an initial dose of 10 mg/kg was followed by a continuous infusion of DPH at a rate of 35 μg/kg/min for periods up to 2 hours. This produced a stable plasma DPH concentration of
11.4 ± 1.2 μg/ml (mean ± SE). Measurements could then be repeated during a prolonged period of stable drug concentration in the plasma.

**AUTOMATICITY**

In a series of experiments, the automaticity of the ventricular specialized conducting system was studied by measuring the time to ventricular escape during vagal stimulation. The distal segment of a crushed vagus was stimulated with pulses 2.0 msec in duration at a frequency of 20/sec for 1 minute. The time for vagal escape was recorded during three stimulations under control conditions and during three stimulation periods after administration of DPH.

The effect of DPH on the ventricular rate of dogs with complete heart block was evaluated in a separate series of experiments. The escape time when ventricular pacing for 10 minutes at a cycle length of 600 msec was discontinued and the rate of spontaneous ventricular rhythm were measured before and after intravenous administration of 5-20 mg of DPH/kg, to estimate the effect of DPH on ventricular automaticity.

**CONDUCTION VELOCITY**

To evaluate linear conduction velocity in right ventricular muscle before and after giving DPH, a technique similar to that of Swain (22) and Lewis and Rothschild (23) was used. A plaque bearing six pairs of electrodes was sutured to the right ventricular outflow tract. The distance between the first two pairs of electrodes was 12 mm and that between each of the subsequent pairs 3 mm. The ventricle was paced at twice the threshold current from the first pair of electrodes and the time at which the rapid spike of activation appeared under each electrode pair was recorded for the basic driving stimulus and after premature excitation. The stimulus also triggered the oscilloscope facilitating the measurement of the time of arrival of responses under each electrode pair. These measurements were repeated after administration of DPH.

**DETERMINATION OF THRESHOLD VOLTAGE IN PURKINJE FIBERS**

Mongrel dogs weighing 10 to 14 kg were anesthetized with intravenously administered pentobarbital sodium (30 mg/kg). The heart was rapidly excised and dissected in oxygenated Tyrode's solution at room temperature. Preparations containing Purkinje fibers and ventricular muscle were dissected from either ventricle and placed in a wax-lined tissue bath. The bath was perfused with Tyrode's solution, gassed with 95% oxygen-5% carbon dioxide, at a constant flow rate of 7 ml/min; the temperature of the perfusate was maintained at 36 ± 1°C. The composition of the Tyrode solution, and the arrangement of the recording system have been described previously (7).

The transmembrane voltage was recorded through 3M KCl-filled microelectrodes inserted into the myoplasm of a Purkinje fiber. Depolarizing current pulses were applied intracellularly through a second microelectrode introduced into the same fiber at a distance of 50-200 μm from the recording microelectrode. The current-passing microelectrode was filled with either 3M KCl or 2.5M Kcitrate. Depolarizing current pulses were applied during diastole; the pulse duration was held constant at 200 msec while the current amplitude was increased stepwise until an extra response occurred, indicating that threshold had been reached (Fig. 3).

**EFFECT OF DPH ON ELECTRODE SYSTEM**

We found that the impedance of the silver electrodes employed in the studies of excitability of the in-situ heart depended on polarity and current strength. The impedance of a silver cathode was an inverse function of the current. This property of silver electrodes, in vivo, was markedly altered after injection of DPH. The impedance of the electrode system at small currents was decreased five- to sixfold after DPH while the impedance at high currents was reduced to half so that the impedance-current relationship was less steep.

Using a burst of 100 Hz alternating current as a stimulus, current, voltage and phase angle were measured simultaneously. Impedance, capacitive reactance, and resistance were calculated assuming no inductive reactance was present. The capacitive reactance component of the impedance was a strong inverse function of current density at the electrode-heart interface and independent of the phase of the cardiac cycle during which the stimulus was applied. The decrease in electrode impedance in the presence of DPH proved to be a property more related to the electrode-electrolyte solution interface than to the excitable cardiac tissue itself; similar changes in the relationship of current to capacitive reactance occurred when electrodes were placed on rabbit kidney and DPH administered intravenously to the rabbit, or when the electrodes were immersed in isotonic saline and DPH added to the saline. Adjustment of the saline pH to 12 in the in-vitro system did not induce these impedance changes. The remarkable effect of DPH on the impedance of the electrode system used to test excitability was unexpected but did not alter the interpretation of our experiments, because current strength was the variable related to the responses obtained.
**Results**

**EFFECT OF DPH ON DIASTOLIC THRESHOLD**

Table 1 summarizes the effect of DPH on diastolic excitability. DPH, 10 mg/kg administered in alkaline saline, decreased the mean amplitude of current pulses, 5 msec in duration, required to stimulate atrial or ventricular muscle during late diastole. Although anodal pulses had to be two to three times the amplitude of cathodal pulses to obtain a response, change in the same direction was obtained with either anodal or cathodal current pulses. The changes in threshold induced by DPH were small but statistically significant ($P < 0.05$). The decrease in diastolic threshold produced by DPH was measurable for 15 to 20 minutes after single intravenous injections.

Diastolic threshold was measured after injections of alkaline saline containing no drug, commercial diluent (10% ethanol, 40% propylene glycol, pH adjusted to 12 with NaOH), and commercial diluent containing DPH. There was no change in diastolic threshold after injecting 5 ml of alkaline saline intravenously into five dogs. Diastolic threshold to monopolar rectangular cathodal and anodal current pulses was determined before and after intravenous injection of 0.2 ml of the commercial diluent for DPH per kg of body weight—the amount used to administer 10 mg/kg of DPH. Table 1 shows that this dose of diluent caused substantial increases in diastolic threshold in both atrium and ventricle. The increase in threshold produced by commercial diluent persisted for 15 to 30 minutes.

When DPH (10 mg/kg) was injected intravenously in commercial diluent (0.2 ml/kg), diastolic threshold decreased in 20 of 32 animals studied. However, the mean changes in this group of animals were not statistically

<table>
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<th>After</th>
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<td>178 ± 47†</td>
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<tr>
<td>Ventricle</td>
<td>5</td>
<td>167 ± 36</td>
<td>178 ± 44</td>
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Values are means ± se. *$P < 0.05$. †$P < 0.01$.  

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significant (Table 1). When intravenous injections of DPH dissolved in commercial diluent were sudden, the arterial pressure fell quickly by an average of 30% and returned to control values within 2 to 4 minutes. Since measurements of diastolic threshold made during this hypotensive period were erratic, this combination was injected slowly over a 5-minute period, which reduced the fall in arterial pressure to less than 10% of control. Figure 2 shows that injection of diluent alone (0.2 ml/kg) elevates the diastolic cathodal ventricular strength duration curve. A subsequent injection of DPH caused the strength-duration relationship to return almost immediately toward control values even though it was dissolved in an additional 0.2 ml/kg of diluent. Previous treatment with atropine (1 mg/kg) and propranolol (0.2 mg/kg) to attenuate any effect mediated through the autonomic nervous system, did not alter the effects of DPH on diastolic excitability.

In four experiments, diastolic threshold was measured with cathodal pulses in ventricular muscle made ischemic by partial occlusion of the left anterior descending coronary artery (2). In each of these experiments, diastolic threshold decreased almost immediately after arterial constriction but increased after injection of 10 mg/kg of DPH in saline.

**EFFECT OF DPH ON THRESHOLD VOLTAGE IN PURKINJE FIBERS**

Transmembrane threshold voltage, measured under control conditions in seven Purkinje fiber preparations, was 70.8 ± 0.86 mv (mean ± se). Threshold voltage was successfully measured before and after exposure to 1 × 10⁻⁷M DPH without losing the impalement of either microelectrode in three preparations (Fig. 3). In these preparations, under control conditions, the following values were found: transmembrane resting voltage 89.7 ± 0.33 mv, action potential amplitude 118.0 ± 1.53 mv, action potential duration 463 ± 4.41 msec, and transmembrane threshold voltage 68.5 ± 0.68 mv. The values after DPH were: resting voltage 89.3 ± 0.67 mv, action potential amplitude 118.7 ± 1.67 mv, action potential duration 430 ± 3.51 msec, and threshold voltage 75.1 ± 0.24 mv. Thus, action potential duration was decreased after DPH by 7% and threshold voltage increased (made more negative) by 10%; each change was statistically significant (P < 0.01). In addition, the amplitude of the intracellular current pulse required to bring the fibers to threshold decreased from control values of 2.97 ± 0.07 × 10⁻⁷ amp to 2.20 ± 0.03 × 10⁻⁷ amp in the presence of DPH; this represented a 26% change and was statistically significant (P < 0.01).

**STRENGTH-INTERVAL RELATIONSHIP**

The strength-interval relationship was evaluated before and after DPH administration in 30 dogs. Except for two dogs that showed very small changes, the strength interval curve shifted to the left after DPH injection,
EFFECTS OF DPH ON EXCITABILITY AND AUTOMATICITY

FIGURE 3

Effect of DPH on threshold voltage of an isolated, perfused Purkinje fiber. In each panel the upper trace records time markers which recur every 100 msec; the second trace, superimposed on the time trace, records the intracellular current being applied to the Purkinje fiber, and the lowest trace records the transmembrane voltage of the Purkinje fiber. Current and voltage calibrations are shown on the right.

A and B: Control conditions. In A, a $2.77 \times 10^{-7}$ amp depolarizing constant current pulse, 200 msec in duration, was applied to the fiber. This pulse depolarized the fiber from $-90.0$ to $-68.0$ mv, but failed to reach threshold voltage. In B, the current pulse amplitude was increased to $2.86 \times 10^{-7}$ amp, the fiber was depolarized to its threshold voltage of $-67.6$ mv, and activation occurred.

C and D: After 30-minute exposure to $1 \times 10^{-7}$M DPH. DPH shortened the action potential duration from 472 to 435 msec. In C, a current pulse $2.15 \times 10^{-7}$ amp in amplitude was applied to the fiber in diastole; the fiber was depolarized from $-88.0$ to $-75.3$ mv without firing. In D, the timing and duration of the pulse were kept constant and its amplitude increased to $2.19 \times 10^{-7}$ amp; the fiber fired when depolarized to $-74.6$ mv (threshold voltage). DPH thus substantially increased the threshold voltage and reduced the current required to stimulate the fiber.

whether or not the drug was given in diluent. The leftward shift in the strength-interval curves was present no matter whether bipolar, stigmatic cathodal, or stigmatic anodal stimuli were used. The effects of DPH on excitability during the relative refractory period are most clearly demonstrated by examination of results obtained with stigmatic anodal or cathodal stimuli. The results obtained with bipolar stimulation are more difficult to interpret since strength interval curves obtained using this method of stimulation represent a composite of anodal and cathodal effects (24, 25).

The effects of DPH on the cathodal strength interval curve can be seen in Figure 4, which shows data from typical experiments in the atrium (A) and ventricle (B). The mean leftward shift of the cathodal strength-interval curve was $17 \pm 3.6$ msec ($P < 0.05$) in the atrium and $24 \pm 4.9$ msec ($P < 0.01$) in the ventricle. After intravenous DPH, it was invariably possible to stimulate either atrium or ventricle earlier in the cardiac cycle than it was before drug injection. The supernormal period, an interval in the relative refractory period when there is a decreased threshold to cathodal current pulses with respect to threshold in diastole, occasionally changed in duration or level of threshold, but these changes were never striking. DPH still shortened the refractory period of both atrium and ventricle when previous treatment with atropine (1 mg/kg), propranolol (0.2 mg/kg), or
A: Atrial strength-interval curve determined with a stigmatic cathodal stimulus of 5 msec in a representative experiment. The control curve was obtained after previous treatment with atropine (1.0 mg/kg) and propranolol (0.2 mg/kg). After atropine and control observations, DPH (10 mg/kg) was injected intravenously, and the strength-interval relationship was reetermined. DPH caused a small decrease in diastolic threshold shortening of the refractory period, after cholinergic and adrenergic blockade.

B: Ventricular strength-interval curve obtained using a stigmatic cathodal stimulus 5 msec in duration. DPH produced no significant change in diastolic threshold but did produce a significant shortening of the refractory period. The smooth rise in threshold current amplitude noted under control conditions was altered after DPH. A hump appeared between the shallow supernormal phase of excitability and the rapidly rising portion of the curve. This hump was carefully defined by measuring threshold current every 0.5 msec over this portion of the curve. The possibility of myocardial activation from the indifferent electrode was eliminated by careful observation of the activation times around each of the stimulating electrodes.

Both were employed to avoid effects related to release of autonomic mediators.

The effects of DPH on the anodal stigmatic strength interval relationship can be seen in Figure 5. The anodal control curves usually showed the features typically seen with this mode of stimulation: (1) diastolic threshold exceeded the cathodal threshold in each case; (2) there was an increase in anodal threshold beginning before the rise in cathodal threshold which had the appearance of a hump; (3) earlier in the cycle, there was a decrease in anodal threshold below that found in diastole, the so-called dip; (4) occasionally, earlier secondary dips were obtained at higher threshold current strengths; and (5) the no-response phenomenon was usually noted (21, 25). After administration of DPH: (1) diastolic threshold usually fell only slightly; (2) the hump often changed in amplitude and duration but these changes were not qualitatively uniform; (3) the level of the "dip" usually increased; (4) the "absolute" refractory period became shorter; and (5) the rise in threshold during the relative refractory period was often steeper (Fig. 5). The mean leftward shift of the anodal strength interval curve was 19 ± 4.2 msec (P < 0.05) in the atrium and 26 ± 4.7 msec (P < 0.01) in the ventricle. Again, these changes after DPH were noted in atrium and ventricle whether or not there had been previous treatment with...
EFFECTS OF DPH ON EXCITABILITY AND AUTOMATICITY

Figure 5
Strength-interval relationship obtained in the ventricle using stigmatic anodal pulses 10 msec in duration. Threshold current amplitude is plotted as a function of the interval in the cardiac cycle. After DPH (10 mg/kg), diastolic threshold was not significantly changed, the refractory period shortened considerably, and the hump and dip became more prominent. The points to the right of each curve (shading) represent ventricular multiple response thresholds obtained before and after DPH. The multiple response threshold was measured several times and ventricular fibrillation threshold once before and after DPH; DPH increased the multiple response threshold from 3 to 16 ma. C.L. = cycle length.

Figure 6
Atrial anodal strength-interval relationship under control conditions and after intravenous injection of commercial diluent (0.2 ml/kg body weight, the amount of diluent ordinarily used to deliver 10 mg/kg of DPH). After administration of diluent, diastolic threshold was elevated and the hump amplitude was attenuated, with no alteration of the level reached by the primary dip in the anodal strength-interval curve. The refractory period, measured under the anode, was prolonged by 30 msec after injection of the diluent. C.L. = drive cycle length.

Atropine (1 mg/kg) and propranolol (0.2 mg/kg) (Fig. 4A). Diluent alone (0.2 ml/kg) shifted both anodal and cathodal atrial strength interval curves to the right by 12 ± 5.2 msec (P < 0.05) and shifted the ventricular cathodal and anodal curves by 18 ± 3.9 msec (P < 0.05) (Fig. 6).

When prolonged intravenous infusion at a rate of 35 μg/kg/min was employed after a loading dose of 10 mg/kg, the plasma level was maintained between 10 and 14 μg/ml (11.4 ± 1.2, mean ± se, n = 12). Under these conditions, the DPH-induced changes in the strength-interval relationship were well maintained. If DPH infusion was stopped, these values returned toward control levels at 1 hour and were not significantly different from control values by the second hour, when plasma levels were 1-3 μg/ml. A second injection of 10 mg/kg at this time invariably moved the anodal and cathodal strength-interval curves leftward again and raised the plasma level to concentrations between 14 and 17 μg/ml.

EFFECT OF DPH ON EFFECTIVE REFRACTORY PERIOD

The values obtained for the effective refractory period are dependent on the amplitude of the test pulse and type of stimulation. Due to the composite nature of...
the bipolar strength interval curve and the observed effects of DPH, (Figs. 4 and 5) stimuli two times threshold gave inconsistent results (24, 25); a consistent shortening of the effective refractory period was found using bipolar test pulses of greater amplitude. If the responses to stigmatic cathodal or anodal stimulation were used, the effective refractory period was unequivocally shortened even with test stimuli of two to four times threshold.

**EFFECT OF DPH ON VENTRICULAR MULTIPLE RESPONSE AND FIBRILLATION THRESHOLDS**

The ventricular or atrial multiple response threshold was determined before and after DPH in ten experiments. Because the criticism has been raised that the multiple response threshold may not always vary in a manner identical to the fibrillation threshold (26), the atrial fibrillation thresholds were measured in eight dogs and ventricular fibrillation thresholds in six dogs before and after DPH. When stimuli were delivered in the vulnerable period, there was, in every case, an orderly progression of responses from a single extrasystole to multiple responses to fibrillation, as stimulus amplitude was increased; the fibrillation threshold was usually 2-4 ma above the multiple response threshold. The vulnerable period for both multiple responses and fibrillation was shifted leftward in every experiment (Fig. 5). The test pulse amplitude necessary to elicit either multiple responses of a comparable number or fibrillation was elevated in 20 of the 24 experiments (Fig. 5). In two experiments there was no rise in ventricular fibrillation threshold and in two others, the vulnerable period could not be located after drug administration. Whether this was due to elevation of the fibrillation threshold or to a temporal shift of the vulnerable period could not be established.

**EFFECT OF DPH ON VENTRICULAR RHYTHM IN DOGS WITH HEART BLOCK**

DPH (5 to 20 mg/kg) was administered in experiments on eight dogs with previously induced complete heart block. When the drug was slowly administered intravenously, arterial pressure was minimally affected, and the rate of the ventricular rhythm was unchanged. Sudden intravenous injections caused decreases in mean systemic arterial pressure of 20 to 25% and a slight increase in the ventricular rate. DPH prolonged the time from cessation of ventricular pacing cycle at a rate of 100/min to the establishment of a stable ventricular rhythm, in five dogs; this time increased from 6 ± 2 sec (mean ± se) to 11 ± 3 msec (P < 0.01).

**EFFECT OF DPH ON VENTRICULAR ESCAPE TIME DURING VAGAL STIMULATION**

Vagal stimulation was performed three times before and after administration of 10 mg/kg of DPH, in seven dogs. In two animals the vagal escape time could not be determined after DPH, since a slow atrial rhythm was the escape mechanism and the atrial escape beats were conducted to the ventricles. In the remaining five dogs, the control ventricular escape time was 15.6 ± 0.6 sec (mean ± se, n = 15); after DPH, the vagal escape time increased 28% to 20.0 ± 1.0 sec (n = 15, P < 0.01). This change was interpreted as a DPH-induced decrease in automaticity in the ventricular specialized conducting system.

**EFFECTS OF DPH ON INTRAVENTRICULAR CONDUCTION**

Conduction time was measured in seven experiments with a multi-contact electrode sutured to the right ventricular outflow tract, using a technique similar to that used by Swain and Weidner (22). The conduction time from the site of stimulation to each of five linearly spaced electrodes 12.5, 15.5, 18.5, 21.5 and 24.5 mm away were measured during ventricular pacing. Conduction time to each electrode in milliseconds was plotted against distance in millimeters in each experiment before and after 10 mg/kg of DPH. The correlation coefficient between conduction time and distance from the site of stimulation was greater than 0.89 in each set of data. The regression of distance on conduction time (apparent conduction velocity) became steeper in each experiment after DPH. The mean conduction velocity in the ventricular muscle of the right ventricular outflow tract before DPH was 0.59 ± 0.03 m/sec (mean ± se) and after DPH, increased to 0.67 ± 0.03 m/sec (P < 0.05). Acceleration of conduction after
DPH administration was also seen when stimuli were placed in the relative refractory period. Swain and Weidner obtained two slopes in the isolated dog heart and attributed the first to conduction in ventricular muscle and the second to conduction in Purkinje tissue. The electrodes in our study were not sufficiently distant from the site of stimulation to estimate conduction velocity in the Purkinje system.

Discussion

Diphenylhydantoin has been shown effective in treating the arrhythmias that follow myocardial infarction, open heart surgery, and digitalis excess (9, 13, 15, 17, 19). The present studies have defined the action of DPH on excitability and automaticity in the canine heart in vivo. It was assumed early in these studies that DPH would exhibit properties like those of quinidine, since this has been the case with all antiarrhythmic drugs previously studied. This hypothesis must, however, be rejected.

Clinical experience with DPH suggests that it differs substantially from quinidine in its effect on fundamental electrophysiological processes in the human heart. DPH does not prolong the QRS interval and shortens the QT interval (17); these observations indicate that the drug does not prolong intraventricular conduction and suggest that it shortens the refractory period. In addition, DPH is ineffective in converting atrial flutter and particularly atrial fibrillation to normal sinus rhythm (10, 17, 18). All these observations are diametrically opposed to clinical observation made on the effects of quinidine or procaine amide.

Automaticity is a fundamental property of many cell types in the atrium and ventricle. Alterations in automaticity have been proposed as a mechanism contributing to a wide variety of clinical arrhythmias (1). This proposal has not been well established because direct observation of the behavior of automatic cells in the ventricular specialized conducting system in man is largely limited to instances of sinoatrial slowing or atrioventricular block. However, correlation of clinical observation with laboratory experiment does strongly suggest that automaticity may play a role in many clinical arrhythmias (1).

Previous work has shown that DPH decreased automaticity in isolated, perfused canine Purkinje fibers (7), markedly decreased the vagal escape time in acetylthrophanthidin intoxicated dogs (20), and had little effect on the ventricular rate of dogs with complete heart block unless the rate had been augmented by previous treatment with deslanoside (8). The present study confirmed the findings of Rosati et al. (8) with regard to the effect of DPH on ventricular rate in dogs with complete heart block. There was no significant change in spontaneous idioventricular rate after intravenous injection of DPH (10 mg/kg). However, we did find that after this dose of DPH there was a significant increase in the time necessary for idioventricular escape rhythms to appear after a period of electrical pacing of the ventricle. This effect is quite similar to our finding that identical doses of DPH increased the vagal escape time by a small but significant amount. This effect of DPH alone on vagal escape time is much less than that seen when DPH is given to animals with enhanced ventricular automaticity due to acetylthrophanthidin (20).

These findings are comparable to those made with DPH during studies of in-vitro canine Purkinje fibers (7), in which DPH decreased automaticity, but seldom even in high concentrations arrested spontaneously firing fibers. In contrast, quinidine and procaine amide appear to depress automaticity more markedly in vivo (7), leading to more prolonged vagal escape time and poststimulation asystole in dogs with heart block (27), and not infrequently cause cessation of automatic firing of Purkinje fibers in vitro (28, 29).

The effects of DPH on our electrode system were of great interest. DPH decreased electrode impedance, either in vivo or during immersion of the electrode system in saline. This effect is probably brought about by changes at the complex interface between the electrode and the surrounding electrolyte.
This allowed the same current to be delivered with a lower pulse voltage but did not cause difficulty in interpreting the results, since current amplitude was the stimulus variable used to characterize excitability.

A diluent is provided with DPH for intravenous use in man because of the low solubility of DPH in water. This diluent, when used alone, increased the diastolic threshold to electrical stimuli and to shift the strength-interval curve to the right, i.e., to prolong the refractory period. Louis et al. (30) showed that the diluent caused bradycardia, ST and T wave changes in the electrocardiogram, hypotension and occasionally ventricular premature contractions in the cat. These authors noted that most of the effects of the diluent were attenuated or abolished by adding DPH. Our studies show that the elevation in diastolic threshold caused by the diluent was lowered toward control by subsequent injection of DPH. This was true even when DPH was dissolved in the diluent.

The changes in diastolic threshold after injecting DPH dissolved in diluent were of small magnitude and inconsistent in direction of change. The inconsistency in effect is probably due to the opposing actions of DPH and the diluent, since DPH in saline consistently caused small but significant decreases in threshold. In any event, changes in diastolic threshold were not large in either atrium or ventricle after either DPH alone or DPH in diluent. This contrasts with the significant increases in diastolic threshold noted after quinidine (21, 32) or procaine amide (33). Moreover, the threshold voltage of Purkinje fibers was increased (made more negative) and the current required to stimulate them was diminished at a concentration previously shown to shorten Purkinje fiber action potential, diminish automaticity and increase the conduction velocity in partially depolarized fibers (7). The effects of DPH on threshold potential are opposite to those found for quinidine by Weidmann (29).

Of further interest was the leftward shift in the strength-interval curve after DPH, particularly in the ventricle. Shortening of the refractory period was accompanied in our experiments by a shortening in the QT interval of the electrocardiogram. This finding is in agreement with the previously observed shortening of QT interval in patients treated with intravenous DPH (17) and with the shortening of isolated Purkinje fiber and ventricular muscle action potentials during exposure to this agent (7). In contrast, quinidine and procaine amide cause lengthening of the refractory period in both atrium and ventricle (21, 32) and prolong the QT interval of the electrocardiogram (34).

DPH, like quinidine and procaine amide, increased the atrial and ventricular fibrillation thresholds. The basis for this time-honored antiarrhythmic assay technique is still not well understood. It is generally agreed, however, that factors tending to cause spontaneous fibrillation also decrease the fibrillation threshold (21, 26, 35). It is also widely held that nonuniformity of refractory period duration and conduction velocity are prerequisites for fibrillation of heart muscle, presumably by favoring the possibility of reentry and fragmented impulse propagation (35). Han and Moe studied many agents known to increase the likelihood of fibrillation and suggested that they all, under selected conditions, could increase the temporal dispersion of recovery of excitability (35). The ability of DPH to elevate fibrillation threshold, particularly in the ventricle, probably is less dependent on shortening of the refractory period than on its ability to increase conduction velocity in partially refractory heart muscle (7). This acceleration of conduction could lead to a decrease in the temporal dispersion of recovery of excitability. In this regard it was noted by Scherf that after DPH treatment, aconitine-induced ventricular tachycardia in dogs could achieve rates as high as 500 without degenerating into fibrillation (5). In a limited number of experiments DPH also elevated ventricular fibrillation threshold when it had been lowered by large doses of ouabain or by ventricular ischemia.

Studies performed with the multicontact electrode indicated an increase in conduction...
velocity in ordinary ventricular muscle after administration of DPH. Although conduction velocity previously has been shown to increase in partially depolarized Purkinje fibers in vitro, after exposure to DPH, conduction velocity in normal Purkinje fibers was not measurably altered (7). We were, therefore, surprised in these studies, to find a measurable increase conduction velocity in ventricular muscle. Conduction may have been somewhat depressed initially in these dogs by the anesthesia and the surgical procedure. Previous studies by Swain and Weidner utilizing a similar method showed a pronounced decrease in ventricular conduction velocity after quinidine (22).

It has been previously suggested that the increase in conduction velocity seen after DPH administration may be an important factor in the antiarrhythmic activity of the drug (7, 36). The primary factors acting to sustain a reentrant pathway are the conduction velocity and the refractory period in the pathway (37). Any agent that accelerates conduction or prolongs refractoriness might be expected to favorably alter a reentrant rhythm. An increase in conduction velocity would increase the minimum necessary length of a reentrant pathway, and could thus abolish an arrhythmia.

DPH then, (1) shortened the refractory period, particularly of ventricular muscle; (2) increased the multiple response and fibrillation thresholds in atrium and ventricle; (3) had little effect on diastolic threshold; (4) slightly enhanced the conduction velocity in ventricular muscle that was stimulated either in diastole or in its relative refractory period; and (5) produced slight decreases in automaticity in the ventricular specialized conducting system in vivo. Many of these effects could be explained by an increase in potassium conductance. Such an increase would decrease the slope of phase 4 depolarization in Purkinje fibers and accelerate the time course of repolarization and might partially explain the beneficial effects of DPH in treatment of digitalis toxicity. The increase in conduction velocity of early premature beats in ventricular muscle could be due to the shortening of the effective refractory period per se, but the fact that conduction velocity was also increased for beats initiated late in diastole, long after full recovery of excitability, makes it likely that the increases in membrane responsiveness previously demonstrated with this drug (7, 35) may play a role. That is, DPH may increase the rate of rise of an action potential in relation to the membrane potential at the time of its initiation. Thus, a fiber excited at any given membrane potential would have a faster rate of depolarization in the presence of DPH, and impulse propagation would be accelerated. The ability of DPH to produce an increase in membrane responsiveness cannot be explained on the basis of increased potassium conductance. It has therefore been suggested that DPH also increases the activity of the sodium-carrying system or increases electrochemical gradient for sodium by enhancing diastolic ion pumping (7, 35). In this regard, Woodbury studied the changes in intracellular sodium concentration and radiosodium turnover in the brains of normal and hyponatremic rats given 40 mg/kg of DPH (38). DPH did not produce clear changes in [Na⁺] of heart muscle from control rats but decreased the intracellular sodium accumulation seen in hyponatremic rats. These experiments suggested that a small increase of sodium pumping in heart muscle occurred in the presence of DPH.

References
4. White, A. W., Jr., Meffinian, R., and Swiss, E. D.: Effects of diphenylhydantoin sodium, glucose, and β-diethy laminoethyl diphenyl pro-


Effects of Diphenylhydantoin on Excitability and Automaticity in the Canine Heart
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