JCI The Journal of Clinical Investigation

The electrophysiologic effects of low and high digoxin concentrations on isolated mammalian cardiac tissue: reversal by digoxin-specific antibody

William J. Mandel, … , J. Thomas Bigger Jr., Vincent P. Butler Jr.

J Clin Invest. 1972;51(6):1378-1387. https://doi.org/10.1172/JCI106933.

Research Article

The effects of digoxin on electrophysiologic properties were evaluated in isolated perfused cardiac tissue. In canine Purkinje fiber (PF)-ventricular muscle (VM) preparations, control measurements, using microelectrode technique, were made of: resting potential (RP), action potential (AP) amplitude, rate of rise, overshoot, duration (APD), membrane responsiveness, conduction velocity (CV), and refractory period. The preparation was then exposed to 1×10^{-7} M digoxin and repeat measurements were carried out every 15 min. At slow (30/min) rates of stimulation APD initially prolonged then markedly shortened. With more rapid stimulation (75 and 120/min) no initial APD prolongation was observed. When stimulated at 75/min, RP and AP rate of rise, amplitude, and CV remained near control values for 60-75 min then rapidly decreased until electrical inexcitability (110±15 min). At that time fibers were perfused with serum containing digoxin-specific antibody (DSA) or one of a group of test solutions. In the preparations exposed to DSA, membrane characteristics improved by 15 min, and by 60 min approximated control values. No beneficial effect was seen with the various test solutions. DSA also reversed digoxin-induced enhanced phase 4 depolarization in PF.

Effective (ERP) and Functional (FRP) refractory periods of rabbit atrioventricular (AV) node preparations were measured in the control state. The tissue was then exposed to 1×10^{-7} M digoxin and refractory period measurements repeated. At a […]

The Electrophysiologic Effects of Low and High Digoxin Concentrations on Isolated Mammalian Cardiac Tissue: Reversal by Digoxin-Specific Antibody

WILLIAM J. MANDEL, J. THOMAS BIGGER, JR., and VINCENT P. BUTLER, JR.

From the Departments of Pharmacology and Medicine, College of Physicians and Surgeons, Columbia University, New York 10032

ABSTRACT The effects of digoxin on electrophysiologic properties were evaluated in isolated perfused cardiac tissue. In canine Purkinje fiber (PF)–ventricular muscle (VM) preparations, control measurements, using microelectrode technique, were made of: resting potential (RP), action potential (AP) amplitude, rate of rise, overshoot, duration (APD), membrane responsiveness, conduction velocity (CV), and refractory period. The preparation was then exposed to 1×10^{-7} M digoxin and repeat measurements were carried out every 15 min. At slow (30/min) rates of stimulation APD initially prolonged then markedly shortened. With more rapid stimulation (75 and 120/min) no initial APD prolongation was observed. When stimulated at 75/min, RP and AP rate of rise, amplitude, and CV remained near control values for 60–75 min then rapidly decreased until electrical inexcitability (110±15 min). At that time fibers were perfused with serum containing digoxin-specific antibody (DSA) or one of a group of test solutions. In the preparations exposed to DSA, membrane characteristics improved by 15 min, and by 60 min approximated control values. No beneficial effect was seen with the various test solutions. DSA also reversed digoxin-induced enhanced phase 4 depolarization in PF.

Effective (ERP) and Functional (FRP) refractory periods of rabbit atrioventricular (AV) node preparations were measured in the control state. The tissue was then exposed to 1×10^{-7} M digoxin and refractory period measurements repeated. At a time when AV conduction prolonged by 20%, associated with marked prolongation of ERP and FRP, DSA or various test solutions were perfused. The prolongation in ERP, FRP, and AV conduction time rapidly returned to normal only in the DSA perfused tissue. It is concluded that DSA has the ability to reverse pronounced toxic electrophysiological effects of digoxin in in vitro cardiac tissue.

INTRODUCTION

Since Withering's discovery of the benefits of Foxglove (1), the digitalis glycosides, as a group, have been among the most useful pharmacologic agents available to the physician. The effects of several digitalis preparations on the electrophysiologic properties of the intact animal have been thoroughly investigated (2–8). However, many variables occur in this setting, such as changes in potassium concentration, pH, or autonomic nervous system activity. Studying isolated cardiac tissue, using the microelectrode technique develop by Ling and Gerard (9), controls these variables so that one may more clearly delineate the electrophysiologic properties of digitalis. Although studies to determine the electrophysiologic properties of digoxin in isolated tissue had not to date been carried out, studies with strophanthin have shown that membrane resistance of cardiac Purkinje fiber was found to initially increase and then decrease (10). This change in membrane resistance was associated with initial action potential lengthening followed by subsequent shortening. Digitalis glycosides can also produce a reduction in rate of rise ($\dot{V}_{max}$) of phase 0, and enhanced automaticity in Purkinje fibers (10, 11).

The clinical use of digitalis is accompanied by frequent development of serious signs of electrical toxicity. An ideal regimen for the satisfactory rapid treatment of

This work was completed during Dr. Mandel's tenure as the Martha Lyon Slater Fellow of the New York Heart Association. Dr. Bigger is Senior Investigator of the New York Heart Association. Dr. Butler is recipient of a Research Career Development Award (HE 11315) from the U. S. Public Health Service.

Presented in part at the Spring Meeting of the American Society for Pharmacology and Experimental Therapeutics, Atlantic City, N. J., 1970 and at the VIth World Congress of Cardiology, London, England, Sept. 1970.

Received for publication 30 August 1971 and in revised form 9 December 1971.

the digitalis-intoxicated patient has to date been elusive (12). Recently, an antibody specific to digoxin (DSA)[1] has been developed (13), and holds promise for treatment of patients so intoxicated (14). The present study had two purposes: first, to delineate the normal and toxic electrophysiologic effects of digoxin on isolated cardiac tissue; and second, to examine the ability of digoxin-specific antibody to reverse the toxic state.

METHODS

Electrophysiological methods. Mongrel dogs (10–20 kg) were anesthetized with sodium pentobarbital, 30 mg/kg, intravenously, and rabbits (1.8–2.5 kg) were stunned by a blow on the head. The hearts were excised quickly and dissected in cool modified Tyrode's solution. Purkinje fiber preparations were obtained from both ventricles and stored in cool, oxygenated Tyrode's solution. AV node preparations were dissected using the method of Paes de Carvalho, de Mello, and Hoffman (15). Preparations were then pinned to the bottom of a wax-lined lucite chamber. The bath was constantly perfused at a flow rate between 5 and 10 ml/min with Tyrode's solution, equilibrated with 95% O_2 and 5% CO_2. Temperature was maintained at 36.0±0.2°C (mean ±SEM). The modified Tyrode's solution contained (in mM): NaCl, 137; KCl, 3.0; NaH_2PO_4, 1.8; CaCl, 2.7; $MgCl_2$, 0.5; dextrose, 5.5; and $NaHCO_3$, 12.0. All solutions were prepared with twice-distilled, deionized water.

Transmembrane potentials were recorded through glass microelectrodes filled with 3 M KCl and having resistances ranging from 15 to 35 megohms. The electrodes were coupled (by Ag-AgCl wire in contact with 3 M KCl) to amplifiers with high input impedance and capacity neutralization (NFl, Bioelectric Instruments, Farmingdale, N. Y.). The amplifier outputs were displayed on a dual beam cathode-ray oscilloscope (Tektronix, Inc., Beaverton, Oreg., RM-565). Surface electrograms were recorded through insulated bipolar silver electrodes.

The maximum rate of rise of phase 0 (V_{max}) of transmembrane potentials recorded from both Purkinje and ventricular muscle fibers was obtained by electronic differentiation as previously described (16). Calibrating time marks repeating at 100- and 500-msec intervals (Tektronix time mark generator, Type 184) were continuously displayed on the oscilloscope trace. The image on the oscilloscope was viewed directly and photographed with a camera (Grass Instrument Co., Quincy, Mass., model C-4) on 35 mm film. The film was enlarged and the magnified images measured.

Stimuli were provided by a series of waveform and pulse generators (Tektronix, Inc., Beaverton, Oreg.). Amplitude and duration of both the basic (S_1) drive and the test stimulus (S_2) could be varied independently as could the interstimulus (S_1-S_2) interval. Stimuli were isolated from ground (Bioelectric Instruments, Farmingdale, N. Y., Type ISA 100 isolation units) and delivered to the tissue through closely placed pairs of insulated Ag wire. S_1 was 3 msec in duration and its amplitude was 1½–2 times threshold; S_2 was 3–5 msec in duration and its amplitude was 3–4 times diastolic threshold.

Tissues were studied in the control state and various times after perfusion with 1×10^{-7} M digoxin. Crystalline digoxin[2] was dissolved in 1 liter of Tyrode's solution to achieve this final concentration. For the canine preparations, intracellular recordings of both VM and PF were obtained in the control state and at 15-min intervals after digoxin perfusion. The following membrane characteristics were determined: resting potential, amplitude, overshoot, rate of rise of phase 0 (dV/dt), action potential duration, effective refractory period, (Purkinje fiber and ventricular muscle), "membrane responsiveness," and conduction velocity (PF only) (17, 18). Refractoriness of both the AV node and the PF-VM junction was measured by stimulating the preparation at a basic drive cycle length, and after every eighth basic beat a premature beat was induced. The coupling interval of the premature beat could be varied widely. AV node preparations were stimulated at the atrial margin and surface electrode recordings made at both the atrial side and distal to the AV node at the bundle of His. Basic responses (A_1 and H_1) and the responses during individual extrasystoles (A_2 and H_2) were recorded. A plot was then constructed comparing the A_1-A_2 interval, on the abscissa, with the H_1-H_2 interval on the ordinate. A similar experimental design was used for measuring refractoriness across the PF-VM-junction. Stimulation was carried out on the VM. The VM_1-VM_2 intervals were plotted on the abscissa and the PF_1-PF_2 intervals plotted on the ordinate (18).

When the preparation showed evidence of severe toxicity, i.e. was essentially electrically inexcitable, perfusion was started with one of the following solutions: (*a*) drug-free Tyrode (70 ml) and serum containing DSA (30 ml) ($K^+ = 3.9$ mEq/liter); (*b*) drug-free Tyrode only (100 ml) ($K^+ = 3.0$ mEq/liter); (*c*) drug-free Tyrode (70 ml), and serum containing antibody to antigens other than digoxin (see below) (30 ml) ($K^+ = 3.9$ mEq/liter); (*d*) drug-free Tyrode (70 ml) and normal rabbit serum (30 ml) ($K = 3.9$ mEq/liter); or (*e*) drug-free Tyrode (70 ml) and normal rabbit gamma globulin (30 ml) ($K^+ = 2.4$ mEq/liter). The pH was 7.36±0.008 for solution (*b*) and 7.38±0.04 for the remaining test solutions.

Additional experiments were performed on preparations having enhanced phase 4 depolarization after perfusion with 1×10^{-7} M digoxin. At a time when phase 4 depolarization became most pronounced, perfusion with one of the above test solutions was instituted.

Immunological methods. Digoxin was conjugated to bovine serum albumin (BSA) and to human serum albumin (HSA) by the periodate oxidation method, as previously described (19). Rabbits were immunized by the injection of BSA-digoxin or HSA-digoxin, 1 mg/ml, in complete Freund's adjuvant mixture, according to an immunization schedule previously outlined (19). Antidigoxin serum was obtained from these rabbits by cardiac puncture or via an ear vein; sheep antidigoxin serum was kindly provided by Dr. D. H. Schmidt. Control serum was obtained from rabbits which had not been immunized and from rabbits which had been immunized with HSA or with purin-6-oyl-HSA in complete Freund's adjuvant mixture (20). Rabbit gamma globulin was obtained as Fraction V powder from Pentex Biochemical (Kankakee, Ill) or prepared from

[1] *Abbreviations used in this paper:* AP, action potential; APD, action potential duration; AV, atrioventricular; BSA, bovine serum albumin; CV, conduction velocity; DSA, digoxin-specific antibody; ERP, effective refractory period; FRP, functional refractory period; HSA, human serum albumin; PF, Purkinje fiber; RP, resting potential; VM, ventricular muscle.

[2] Kindly supplied by Dr. Stanley Bloomfield of Burroughs Welcome & Co., Tuckahoe, N. Y.

normal rabbit serum by a sodium sulfate precipitation method (21). The titers of DSA were determined by the dextran-coated charcoal method as described elsewhere (22, 23). DSA titers are expressed as the highest serum dilutions, 1 ml of which is capable of binding 50% of the added digoxin-^{3}H (32 ng). Sera from nonimmunized rabbits (normal rabbit sera) and sera obtained from rabbits immunized with antigens unrelated to digoxin exhibited no binding of digoxin-^{3}H at a 1:20 dilution. In contrast, the titers of sera containing DSA ranged from 1:800 to 1:3200 (Fig. 1).

RESULTS

Normal electrophysiologic effects

Membrane characteristics. Isolated canine papillary muscle-Purkinje fiber preparations were exposed to digoxin, 1×10^{-7} M, and serial evaluations of membrane characteristics obtained. Fig. 2 shows (as a function of time) the effects of digoxin on amplitude, rate of rise of phase 0, and effective refractory period. These preparations were stimulated at 75 beats/min (cycle length 800 msec) and records obtained until electrical inexcitability occurred.

Panel A of Fig. 2 shows the results otbained for Purkinje fibers in 10 experiments. V_{max} decreased pro-

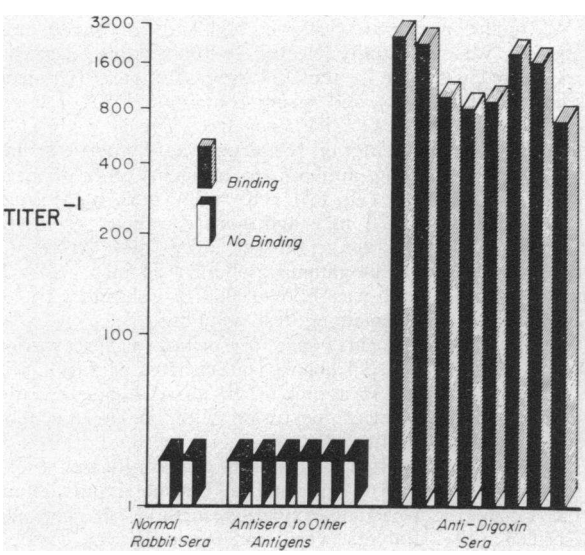

FIGURE 1 Binding of digoxin-^{3}H by anti-digoxin sera. On the abscissa are the three sera subgroups: (*a*) normal rabbit sera; (*b*) antisera to antigens other than digoxin; (*c*) antidigoxin sera. The ordinate displays the titer level (highest dilution capable of binding 16 ng digoxin/ml of serum dilution). The open bars, to the left of the graph, demonstrate the absence of digoxin-^{3}H binding at a 1:20 dilution for both the normal rabbit sera and rabbit antisera to antigens other than digoxin. At the right, the filled bars represent the titers of digoxin-specific antisera, which are expressed as the highest serum dilutions, 1 ml of which is capable of binding 50% of the added digoxin-^{3}H (32 ng). Titers ranged from 1:800 to 1:3200.

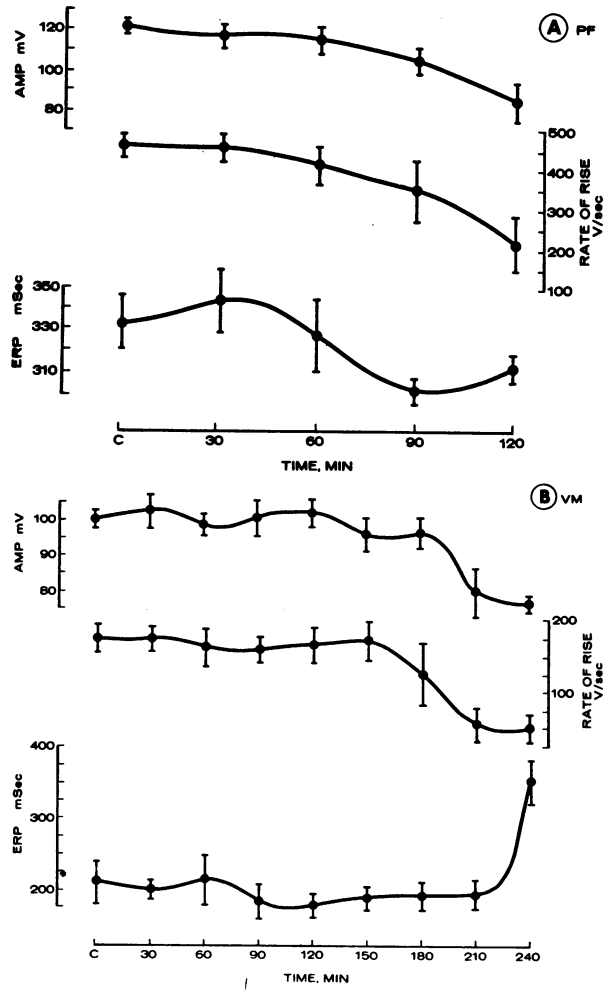

FIGURE 2*A* Effect of 1×10^{-7} M digoxin on transmembrane characteristics of PF. Time in minutes is plotted on the abscissa. C indicates the control values, subsequent values plotted at 30, 60, 90, and 120 min after beginning exposure to digoxin. The vertical axis has scales for amplitude (amp) in millivolts and effective refractory period (ERP), in milliseconds, to the left of the panel. The scale for rate of rise of phase 0 (in volts per second) is seen to the right of the figure. Note the progressive decrease in both amplitude and rate of rise and the triphasic response in ERP. All values obtained at 90 and 120 min were statistically different from control ($P < 0.001$; *t* test for paired samples).

FIGURE 2*B* Effect of 1×10^{-7} M digoxin on transmembrane characteristics of VM. Scales on the ordinate are identical to panel A. The abscissa shows the control period (C) and time in minutes after exposure to digoxin. As with PF, there is a progressive decrease in both amplitude and rate of rise. The most striking change in ERP is the marked prolongation occurring at 240 min. All values for amplitude and dV/dt obtained at 210 and 240 min were statistically different from control ($P < 0.001$; *t* test for paired samples). However, for ERP determinations, only the value at 240 min was statistically significant ($P < 0.01$; *t* test for paired samples).

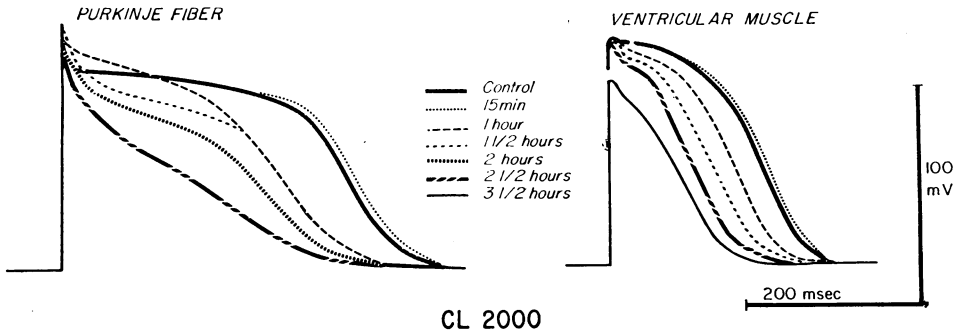

FIGURE 3 Effect of digoxin on AP configuration in PF and VM. Electrical stimuli were applied at a cycle length of 2000 msec (30/min). The figure represents reproduced and superimposed action potentials from the control state and varying periods, from 15 to 210 min, after 1×10^{-7} M digoxin exposure. Note the slight prolongation in APD at 15 min in both PF and VM. This is followed by progressive AP shortening primarily affecting phase 2.

gressively from a control value of 465±25 v/sec (mean ±SEM) to 210±75 v/sec at 120 min of drug exposure. A progressive decrease in AP amplitude from 122±2 mv in the control state to 85±9 mv occurred at 120 min of drug exposure. The effective refractory period (ERP), however, demonstrated a triphasic response. There was an initial, slight prolongation seen at 30 min (345±10 msec) as compared to the control values (333±14 msec). This was followed by shortening with minimum values seen at 90 min (300±6 msec) followed by slight increase seen at 120 min (312±6 msec). Shortly beyond 120 min of digoxin exposure the tissues were essentially inexcitable (see Fig. 4).

In panel B, similar data is shown for ventricular muscle (eight experiments). VM, in contrast to PF did not become electrically inexcitable for at least 240 min (see Fig. 4). As with Purkinje fibers, there was a progressive decrease in amplitude and $\dot{V}_{max}$ as a function of exposure time to digoxin. $\dot{V}_{max}$ decreased progressively from a maximum of 179±16 v/sec during the control period to 53±14 v/sec after 240 min of drug exposure. Quantitatively, similar findings were noted with AP amplitude in VM, with a lowest phase 0 voltage (75±2 mv; control, 100±3 mv) noted (+240 min) just before electrical inexcitability. The ERP in VM demonstrated an initial slight shortening with a minimum value seen at 120 min (183±20 msec). Just before electrical inexcitability (+240 min), there was a pronounced increase in ERP to 350±30 msec.

Action potential configuration and duration. Changes in AP morphology after digoxin exposure were examined in 15 experiments. The experimental records obtained at a slow rate of stimulation (30/min) demonstrated an initial prolongation in duration of 4±0.4% due to an increase in the duration of phase 2. This prolongation was most marked 15 min after digoxin perfusion. Subsequently, there was a progressive decrease in APD, which was associated with an abbreviation of phase 2 as well as an increase in its slope. The initial prolongation in APD seen in this experiment was only apparent at a slow drive rate; more rapid rates of stimulation (75 and 120/min), produced only the progressive shortening in APD. In Fig. 3, PF and VM action potentials from a typical experiment are shown in the control state. Superimposed upon these control traces are the action potentials recorded at various times after exposure to 1×10^{-7} M digoxin. In VM, digoxin had effects similar to those seen in PF. An initial prolongation and subsequent shortening of APD were similar in magnitude and followed much the same time course in each fiber type. Amplitude, rate of rise, and APD, in both PF and VM, were maintained until a significant decrease in RP was noted and then changes in these variables were observed.

Survival time. Serial action potentials were recorded in 19 experiments after exposure to 1×10^{-7} M digoxin. The PF-papillary muscle preparations were continuously stimulated at cycle lengths 500, 800, and 2000 msec and records obtained until the cell became electrically inexcitable. In each fiber type, time to electrical inexcitability was related to the rate of stimulation, the slowest drive rate (30/min, cycle length 2000 msec) being associated with the longest survival time. In addition, there was a marked disparity in the survival times noted between Purkinje and papillary muscle fibers with PF developing toxicity much earlier than VM (Fig. 4).

Purkinje fiber conduction velocity. In five experiments, the effect of prolonged perfusion with 1×10^{-7} M digoxin on conduction velocity in linear PF preparations was studied. All preparations were stimulated at 75/min (cycle length 800 msec) and after control values were obtained, the tissue was perfused with digoxin; recordings were made every 15 min until electrical inexcitability occurred. In this experimental group, significant changes in AP amplitude, and rate of rise were only seen after

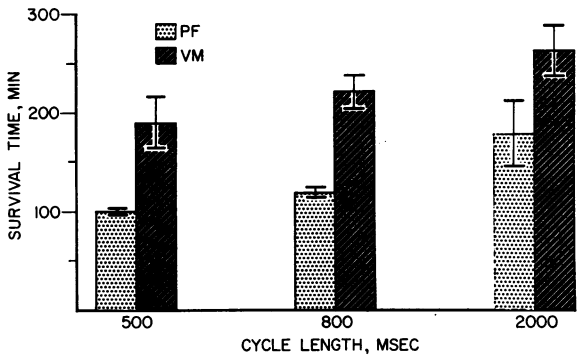

FIGURE 4 Survival time of Purkinje and ventricular muscle fibers in 1×10^{-7} M digoxin. PF-VM preparations were driven at cycle lengths of 500 (120/min; six experiments), 800 (75/min; eight experiments), or 2000 msec (30/min; five experiments) until electrical inexcitability and their survival times plotted on the ordinate in minutes. The cross-hatched bars represent results obtained in VM and the stippled bars represent results obtained in PF. Note (*a*) the progressive increase in survival time in both tissue types as the drive rate is decreased; (*b*) the highly significant difference in survival time between the VM and PF at any given drive cycle length.

90 min of digoxin perfusion. It was not, however, until 120 min of perfusion that the decrease in amplitude and rate of rise produced a significant decrease in the linear conduction velocity. The amplitude at this time was 85±9.0 mv, dV/dt 210±75 v/sec and overshoot 16±4.2 mv. The results of a typical experiment are shown in Fig. 5.

Purkinje fiber-papillary muscle junction. The effect of 1×10^{-7} M digoxin exposure on conduction across the PF-papillary muscle junction was studied in seven preparations. The ERP (shortest $VM_1 - VM_2$ interval) and the FRP (the shortest $PF_1 - PF_2$ interval) were measured in the control state and every 30 min until electrical inexcitability occurred. After digoxin exposure, there was a progressive decrease in both the ERP and FRP of the junction. This was in contrast to the $VM_1 - PF_1$ interval which was noted to increase progressively to a maximum of 36±7 msec (control, 3±0.5 msec). The results of a typical experiment are shown in Fig. 6.

Membrane responsiveness. In five preparations membrane responsiveness curves were obtained in the control state and every 30 min after perfusion was begun with 1×10^{-7} M digoxin. There was no significant change in membrane responsiveness demonstrated up to 60 min after digoxin perfusion was begun. However, by 90 min there was a minimum decrease and at 120 min a highly significant decrease in V_{max} with no significant shift in the responsiveness curve. The results of a typical experiment are shown in Fig. 7.

Reversal of digoxin toxicity by digoxin-specific antibody

Purkinje fiber. In 15 PF preparations 1×10^{-7} M digoxin enhanced phase 4 depolarization. After pronounced phase 4 depolarization was noted, five preparations were then perfused with DSA in drug-free Tyrode's solution. Recordings were made every 2 min until the AP configuration stabilized. In each preparation treated with DSA, phase 4 depolarization was abolished and normal transmembrane voltage characteristics reestab-

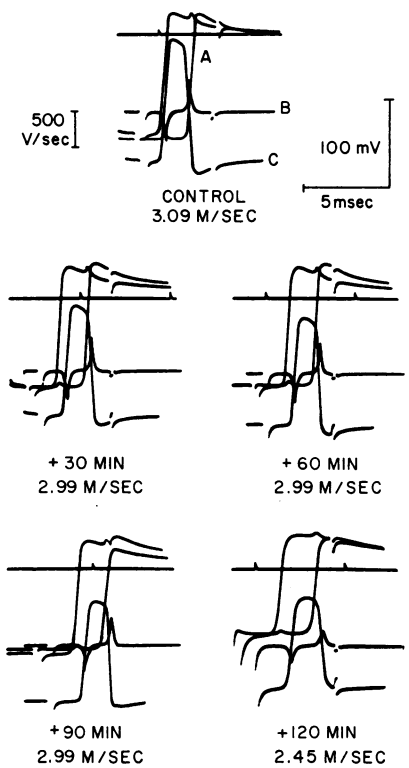

FIGURE 5 Effect of digoxin on CV in linear strands of PF. CV was measured in the control state and at 30, 60, 90, and 120 min after exposure to 1×10^{-7} M. The top panel shows a record obtained in the control state with a voltage, time, and rate of rise calibration. The upper horizontal line displays time marks which recur every 5 msec. Line A shows phase 0 of the cells from the proximal and distal PF at a rapid oscilloscope sweep speed. V_{max} of the proximal and distal cells is shown in line B, and line C shows phase 0 of these same two cells after the signal has been led through a differential amplifier. CV is calculated by dividing the distance between the two recording sites (measured with an occular micrometer) by the time interval between phase 0 of the two upstrokes (measured at the instant of V_{max}). Note that the CV remains constant until 120 min of digoxin exposure. At 120 min, the CV fell by 21% with both a diminished V_{max} (−49%) and AP amplitude (−27%).

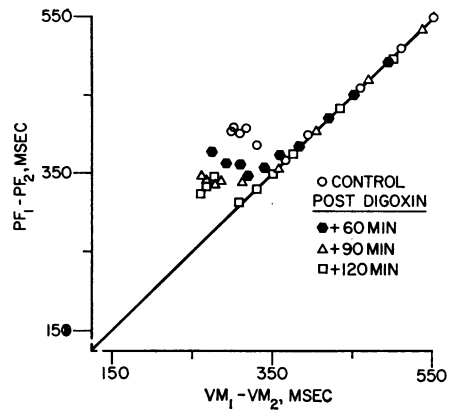

FIGURE 6 The effects of 1×10^{-7} M digoxin on refractoriness at the PF-VM junction. On the abscissa are plotted the VM_1-VM_2 intervals in milliseconds and on the ordinate are plotted the PF_1-PF_2 responses in milliseconds. In the control period the ERP was 295 msec and the FRP was 363 msec. There was a decrease in both of these variables after exposure. The ERP was 268 after 60 min, 266 after 90 min, and 254 after 120 min of exposure to digoxin. The FRP showed a somewhat similar response: 342 after 60 min, 332 after 90 min, and 308 after 120 min of digoxin exposure.

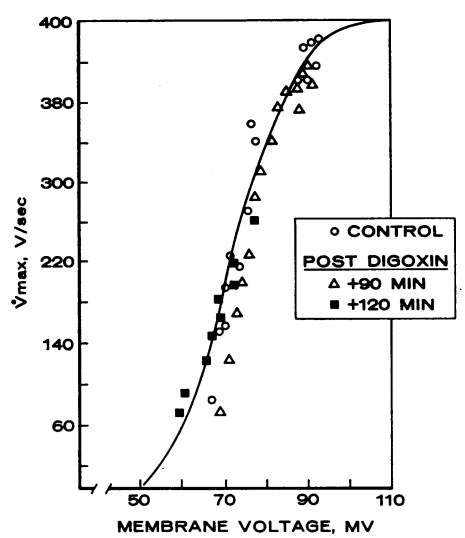

FIGURE 7 Effect of 1×10^{-7} M digoxin on membrane responsiveness. The maximum rate of phase 0 depolarization (V_{max}) in volts per second is plotted (ordinate) as a function of the membrane voltage at the moment of activation in millivolts (abscissa). Control measurements (unfilled circles) are plotted as well as those made after 90 min (unfilled triangles) and 120 min (filled squares) of digoxin exposure. No significant change in peak V_{max} or configuration of the curve was noted after 60 min of drug exposure. After 90 min a slight decrease in peak V_{max} is seen but only after 120 min is there a dramatic decrease in peak V_{max} (C 435 v/sec; +120 min 220 v/sec) without any shift in the curve on its voltage axis.

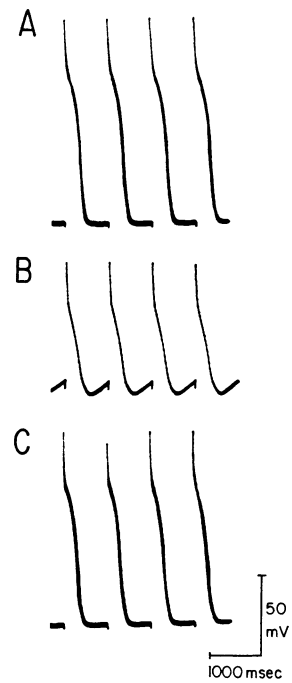

FIGURE 8 Reversal of digoxin-enhanced phase 4 depolarization by DSA. Panel A shows a record obtained in the control state with the fiber being stimulated at a rate of 75/min. An amplitude calibration in millivolts and a time calibration in milliseconds is shown in the lower right hand portion of the figure. Panel B shows the same driven cell after 1 hr of exposure to 1×10^{-7} M digoxin. Spontaneous phase 4 depolarization and diminished amplitude of the AP are evident. Panel C was obtained 10 min after exposure to DSA in drug-free Tyrode. In the same cell driven at 75/min, phase 4 depolarization is abolished and normal transmembrane voltage characteristics were established.

lished within a 15 min period. A typical result employing serum containing DSA is shown in Fig. 8.

In the remaining preparations, with similar enhancement of phase 4 depolarization by digoxin, various control solutions were then perfused: (*a*) drug-free Tyrode (n = 5), (*b*) drug-free Tyrode and hyper-immune serum (n = 5), (*c*) drug-free Tyrode and normal rabbit serum (n = 5), and (*d*) drug-free Tyrode and normal rabbit gamma globulin (n = 5). In no instance was abolition of this enhanced phase 4 depolarization demonstrated by any of these control solutions even though digoxin was not used. In addition, during the period of observations, no significant effect on other AP characteristics was noted.

Another series of 15 experiments was carried out to test the ability of DSA to reverse digoxin-induced electrophysiologic toxicity. PF preparations were exposed to 1×10^{-7} M digoxin until the fibers were nearly or actually inexcitable by extrinsic electrical stimuli. At

this point in time, DSA in drug-free Tyrode or one of the control solutions was used to perfuse the preparation and events followed every 5 min until stable. In all instances, perfusion with DSA-Tyrode's solution was associated with slow return to or near control AP characteristics. None of the other control solutions demonstrated any significant restorative effect. The time-course of events of a typical experiment using DSA-Tyrode's solution are shown in Fig. 9.

AV Node. The final group of experiments were designed to test the ability of DSA to reverse digoxin-induced AV conduction delay. AV node preparations from 25 rabbits were exposed to 1×10^{-7} M digoxin after the ERP (shortest $A_1 - A_2$ interval conducted to the His bundle) and FRP (shortest $H_1 - H_2$ interval) were determined in the control state. At a time when $A_1 - H_1$ interval had increased by at least 20% above control, the preparation was then perfused with serum containing DSA or one of the various control solutions. In the five preparations exposed to the DSA-Tyrode's solution, there was prompt (15±2 min) return of AV conduction to normal. In similar preparations, studied at a time when a similar prolongation of AV conduction was induced by digoxin, no such decrease in AV conduction time, ERP, or FRP was noted when the tissue was perfused with any of the previously described test solutions (Table I).

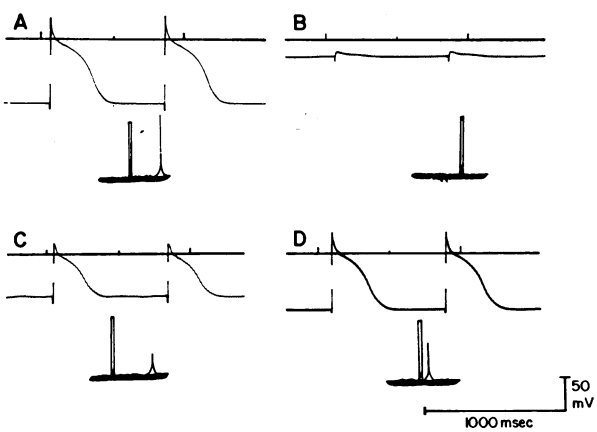

FIGURE 9 Reversal of digoxin-induced toxicity in PF. Panel A displays the AP and, below, $\dot{V}$ of phase 0 and a calibration (500 v/sec) obtained by electronic differentiation. Amplitude time calibrations are shown in the lower right hand portion of the figure. Panel B shows the same cell 120 min after exposure to 1×10^{-7} M digoxin. At this point in time, DSA in drug-free Tyrode was applied. RP has decreased and the cell is essentially electrically inexcitable. Panel C shows the same cell 15 min after DSA perfusion. There is now a return toward normal of the cell's electrical characteristics. Panel C shows the same cell, 60 min after DSA exposure. The cell shows even further return toward normal in its electrical characteristics.

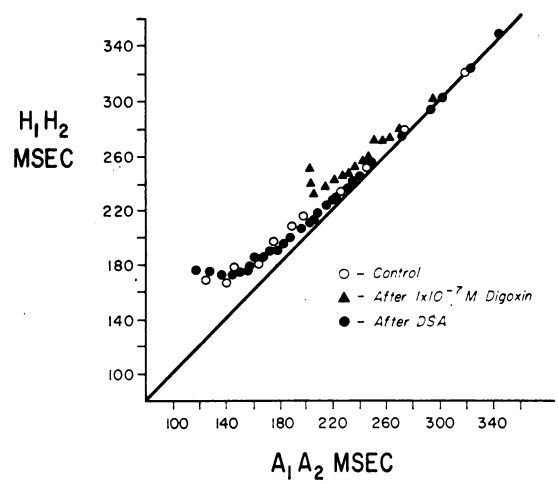

FIGURE 10. Effect of digoxin on refractoriness of the AV node, and its reversal by DSA. The A_1-A_2 interval is plotted on the abscissa and the H_1-H_2 response is plotted on the ordinate. The basic cycle length is 500 msec. The control observations (unfilled circles) demonstrate an ERP of 125 msec and FRP of 166 msec. After 45 min exposure to 1×10^{-7} M digoxin (filled triangles), the ERP was 203 msec and FRP 229 msec. DSA was applied and after 10 min refractory periods measured (filled circles); the ERP (118 msec) and FRP (172 msec) shortened to values which were not significantly different from the control.

The results of a typical experiment utilizing DSA are shown in Fig. 10.

DISCUSSION

Previous studies have described the effects of several digitalis compounds on the electrophysiologic characteristics of cardiac tissue (2–8, 10, 11, 24–26). There has, however, been no detailed electrophysiologic investigation of the effects of digoxin on isolated cardiac tissue. The results described in the initial portion of this study demonstrate that the electrophysiologic properties of digoxin in isolated cardiac tissue are qualitatively similar to previously studied glycosides. Specifically, digoxin induced a time-dependent decrease in AP, amplitude, and rate of rise which was noted after 60 min in PF and 180 min in VM. The progressive decrease in these variables was paralleled by a progressive decrease in APD. The time-course of these changes cannot, in absolute terms, be compared with previous studies with ouabain and strophanthin (10, 11) because of differences in molar concentrations. However, the time-course differential obtained with digoxin between the more sensitive PF and the less sensitive VM is similar to that noted for ouabain (11). In addition, survival time after ouabain and strophanthin exposure has been found to be inversely related to the stimulation frequency. This study has shown that a quantitatively similar relationship was

TABLE I

AV refractory period	Test solutions: DSA-Tyrode	Drug-free tyrode	Hyper-immune serum	Normal rabbit serum	Rabbit gamma globulin
			msec		
Control ERP	132±4.8	132±4.8	132±4.8	132±4.8	132±4.8
FRP	170±8.4	170±8.4	170±8.4	170±8.4	170±8.4
	n = 25	n = 25	n = 25	n = 25	n = 25
Post-digoxin ERP	199±9.7	199±9.7	199±9.7	199±9.7	199±9.7
FRP	235±12.2	235±12.2	235±12.2	235±12.2	235±12.2
	n = 25	n = 25	n = 25	n = 25	n = 25
	$P < 0.001$*	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
15 min after test serum ERP	130±6.7	203±6.7	207±7.6	200±9.9	204±7.9
FRP	175±9.6	235±15.1	240±8.9	242±12.8	242±16.1
	n = 5	n = 5	n = 5	n = 5	n = 5
	$P < 0.001$	NS	NS	NS	NS

* *t* test for paired samples, mean ±SEM.

found with digoxin (10, 11, 26). Serial studies on the relationship of peak $\dot{V}_{max}$ to digoxin exposure initially demonstrated little change in either PF or VM. However, with continued exposure, there eventually was a decrease in peak $\dot{V}_{max}$. Associated with this finding was a corresponding decrease in amplitude and conduction velocity. The CV decrease would be anticipated as a corresponding significant decrease in its major determinants; AP amplitude and rate of rise of phase 0 were observed (27). In addition, serial measurements of membrane responsiveness were also done. This determination, which is felt to represent the availability of the sodium-carrier system (28), was initially little altered by digoxin exposure. Slight change was noted in V_{max} at 90 min, and by 120 min a marked decrease in V_{max} was seen without any shift in the curve on its voltage axis. Therefore, digoxin has no specific effect on the sodium-carrier system.

As previously described for strophanthin, at slow stimulation rates (30/min), APD in VM initially increased followed by progressive decrease; with a more rapid rate of stimulation (60/min) only AP shortening was observed (10). The present study showed similar findings for digoxin in both VM and PF. Configurational changes associated with shortening of APD demonstrated an initial decrease in the plateau or phase 2 followed by associated change in the slope of phase 3. It is as yet unclear what ionic species are responsible for the plateau phase of the AP. Prior studies have commented on the contribution of Na^+, K^+, Cl^+, Ca^{++} ions to the maintenance of phase 2 (29–32). Repolarization of phase 3 has, in large part, been related to a change in gK (30, 33, 34). The findings in this study suggest that an increase in gK may be the predominant factor for digoxin-induced changes in AP configuration.

Studies by Müller have offered partial confirmation by showing that even low concentrations of ouabain are associated with a decrease in intracellular K^+ (24). Recent studies by Polimeni and Vassalle have suggested that the toxic effects of ouabain are related to its ability to compete with K^+ at the outer layer of the cell membrane (26). This would result in inhibition of K^+ influx and Na^+ efflux. These authors, in addition, demonstrated that the apparent reason for the time disparity between ouabain toxicity in VM as compared to PF is that it requires 3 times as much ouabain to reduce K^+ influx in VM as compared to PF.

Studies on the ERP of VM demonstrated minimal shortening except during overt toxicity when marked prolongation was noted. In contrast, PF ERP was noted initially to prolong slightly, followed by pronounced shortening. Just before inexcitability, the ERP began to increase slightly.

Studies undertaken to show effects of digoxin on the refractiveness across the PF-VM junction demonstrated progressive shortening of the effective and functional refractory periods as drug perfusion was continued over a 120 min period. These findings are in sharp contrast to the prolongation of the effective and functional refractory period of the AV node by digitalis glycosides. These observations demonstrate that, in spite of recent studies suggesting a similarity between the physiologic significance of VM-PF junction and the AV node, the response of these two sites to digoxin is clearly dissimilar (35). In our studies refractoriness decreased across the VM-PF junction after digoxin, while conduction time

increased. Although the explanation for this response is unclear, this response almost certainly contributes to the development of reentrant ventricular arrhythmias in digitalis toxicity (36).

In the second portion of this study, the efficacy of DSA in reversing digoxin-induced electrophysiologic toxicity was tested. DSA was able to reverse digoxin-induced electrical inexcitability, marked phase 4 depolarization in PF, as well as reverse digoxin-induced prolongation in AV nodal refractoriness. The results of these studies demonstrate that, in severely intoxicated PF-VM preparations, DSA perfusion resulted in return to near normal electrophysiologic characteristics within 60 min. This time sequence is in keeping with recent results with PF regeneration time after prolonged cooling (37). The more rapid reversal after DSA perfusion in the AV node preparation presumedly reflects the degree of digoxin toxicity (i.e., degree of inhibition of the Na^+K^+ ATPase system, see below).

Repke, Schwartz et al., and Akera et al. have proposed that cell membrane adenosine triphosphatase may be the pharmacologic receptor site for the digitalis glycosides (38–40). Repke has further postulated that glycoside binding to the enzyme may occur as a result of hydrogen binding from the lactone ring carbonyl moiety to a protein receptor (41). Furthermore, Okita has stated that digitalis glycosides are not firmly bound, but, in fact, the binding to the receptor site is reversible (42). This latter finding is in keeping with the findings of this study which showed reversibility of digoxin toxicity in several test systems.

The Na^+, K^+-dependent ATPase is the only enzymatic system consistently affected by digitalis glycosides (39, 43). Although the precise location of a possible ATPase receptor is as yet unknown (39), cell membrane and T-system are candidate sites (38, 41). Schwartz, Allen, and Harigaya also comment that potency is directly dependent on drug-enzyme exposure (39). This is in keeping with our findings of progressive changes in AP characteristics during continuous monitoring. This suggests that slow equilibrium is conceivably due to relatively inaccessible receptor sites or slow intracellular ion concentration changes after Na^+K^+ ATPase is inhibited by a constant per cent.

Although the exact mechanism of glycoside-induced positive inotropy is not clarified, increase in internal sodium concentration seemingly permits an increase of calcium ions at the active contractile site and enhanced tension development (44). The inhibition of active sodium pumping via diminished Na^+K^+, ATPase activity secondary to digitalis glycosides may be the major factor. The changes in internal milieu and ionic fluxes must be the major determining factors in the observed changes in AP characteristics.

It seems clear from our present observations that DSA has the ability to reverse severe manifestations of digoxin toxicity in isolated cardiac tissue. The exact site of activity of digoxin or its specific antibody is uncertain from these studies, but because of these above observations, a receptor site on or near the membrane surface is conceivable.

ACKNOWLEDGMENTS

Supported in part by U. S. Public Health Service Grants HE 12738, HE 10608, and HE 05741, and in part by Grants-in-Aid from the New York Heart Association and the American Heart Association (69-824).

REFERENCES

1. Withering, W. 1785. An Account of the Foxglove and Some of its Medical Uses: With Practical Remarks on Dropsy and Other Diseases. C. G. J. and J. Robinson, London.
2. Méndez, C., and R. Méndez. 1953. The action of cardiac glycosides on the refractory period of heart tissues. *J. Pharmacol. Exp. Ther.* **107**: 24.
3. Moe, G. K., and R. Méndez. 1951. The action of several cardiac glycosides on conduction velocity and ventricular excitability in the dog heart. *Circulation.* **4**: 729.
4. Méndez, C., and R. Méndez. 1957. The action of cardiac glycosides on the excitability and conduction velocity of the mammalian atrium. *J. Pharmacol. Exp. Ther.* **121**: 402.
5. Krueger, E., and K. Unna. 1942. Comparative studies on the toxic effects of digitoxin and ouabain in cats. *J. Pharmacol. Exp. Ther.* **76**: 282.
6. Farah, A., and T. A. Loomis. 1958. The action of cardiac glycosides on experimental atrial flutter. *Circulation.* **2**: 742.
7. Méndez, C., J. Aceves, and R. Méndez. 1961. The antiadrenergic action of digitalis on the refractory period of the A-V transmission system. *J. Pharmacol. Exp. Ther.* **131**: 199.
8. Fisch, C., K. Greenspan, S. B. Knoebel, and H. Feigenbaum. 1964. Effect of digitalis on conduction of the heart. *Progr. Cardiovasc. Dis.* **6**: 343.
9. Ling, G., and R. W. Gerard. 1949. The normal membrane potential of frog sartorius fibers. *J. Cell. Comp. Physiol.* **34**: 383.
10. Kassebaum, D. G. 1963. Electrophysiological effects of strophanthin in the heart. *J. Pharmacol. Exp. Ther.* **140**: 329.
11. Vassalle, M., J. Karis, and B. F. Hoffman. 1962. Toxic effects of ouabain on Purkinje fibers and ventricular muscle fibers. *Amer. J. Physiol.* **203**: 433.
12. Mason, D. T., J. F. Spann, Jr., and R. Zelis. 1969. New developments in the understanding of the actions of the digitalis glycosides. *Progr. Cardiovasc. Dis.* **11**: 443.
13. Butler, V. P., Jr., and J. P. Chen. 1967. Digoxin-specific antibodies. *Proc. Nat. Acad. Sci. U. S. A.* **57**: 71.
14. Schmidt, D. H., and V. P. Butler, Jr., 1971. Reversal of digoxin toxicity with specific antibodies. *J. Clin. Invest.* **50**: 1738.
15. Paes de Carvalho, A., W. C. de Mello, and B. F. Hoffman. 1959. Electrophysiological evidence for specialized fiber types in rabbit atrium. *Amer. J. Physiol.* **196**: 483.
16. Bigger, J. T., Jr., A. L. Bassett, and B. F. Hoffman.

1968. Electrophysiological effects of diphenylhydantoin on canine Purkinje fibers. *Circ. Res.* **22**: 221.
17. Bigger, J. T., Jr., and W. J. Mandel. 1970. Effect of lidocaine on the electrophysiologic properties of ventricular muscle and purkinje fibers. *J. Clin. Invest.* **49**: 63.
18. Bigger, J. T., Jr., and W. J. Mandel. 1970. Effect of lidocaine on conduction in canine Purkinje fibers and at the ventricular muscle-Purkinje fiber junction. *J. Pharmacol. Exp. Ther.* **172**: 239.
19. Smith, T. W., V. P. Butler, Jr., and E. Haber. 1970. Characterization of antibodies of high affinity and specificity for the digitalis glycoside digoxin. *Biochemistry.* **9**: 331.
20. Butler, V. P., Jr., S. W. Tanenbaum, and S. M. Beiser. 1965. A study of cross-reactivity of antipurin-6-oyl serum with deoxyribonucleic acid (DNA). *J. Exp. Med.* **121**: 19.
21. Straus, A. J. L., B. C. Seegal, K. C. Hus, P. M. Buckholder, W. L. Nastuk, and K. E. Osserman. 1960. Immunofluorescence demonstration of a muscle binding, complement-fixing serum globulin fraction in myasthenia gravis. *Proc. Soc. Exp. Biol. Med.* **105**: 184.
22. Herbert, V., K. S. Lau, C. W. Gottlieb, and S. W. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**: 1375.
23. Schmidt, D. H., and V. P. Butler, Jr. 1971. Immunological protection against digoxin toxicity. *J. Clin. Invest.* **50**: 866.
24. Müller, P. 1965. Ouabain effects on cardiac contraction, action potential, and cellular potassium. *Circ. Res.* **17**: 46.
25. Watanabe, Y., and L. S. Dreifus. 1966. Electrophysiological effects of digitalis on A-V transmission. *Amer. J. Physiol.* **211**: 1461.
26. Polimeni, P. I., and M. Vassalle. 1971. On the mechanism of ouabain toxicity in Purkinje and ventricular muscle fibers at rest and during activity. *Amer. J. Cardiol.* **27**: 622.
27. Hoffman, B. F., and P. F. Cranefield. 1960. Electrophysiology of the Heart. McGraw-Hill Book Company, Inc., New York.
28. Weidmann, S. 1955. The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *J. Physiol. (London).* **127**: 213.
29. Dudel, J., K. Peper, R. Rüdel, and W. Trautwein. 1967. The dynamic chloride component of membrane current in Purkinje fibers. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **295**: 197.
30. Dudel, J., K. Peper, R. Rüdel, and W. Trautwein. 1966. The potassium component of membrane current in Purkinje fibers. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **296**: 308.
31. Dudel, J., K. Peper, and W. Trautwein. 1966. The contribution of Ca^{++} ions to the current voltage relation in cardiac muscle (Purkinje fibers). *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **288**: 262.
32. Reuter, H. 1967. The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *J. Physiol. (London).* **192**: 479.
33. Peper, K., and W. Trautwein. 1968. A membrane current related to the plateau of the action potential of Purkinje fibers. *Pfluegers Arch. Gesamte Physiol. Menchen Tiere.* **303**: 108.
34. Noble, D., and R. W. Tsein. 1968. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol. (London).* **195**: 185.
35. Myerburg, R. J. 1971. The gating mechanism in the distal atrioventricular conducting system. *Circulation.* **43**: 955.
36. Wallace, A. G., and R. J. Mignone. 1966. Physiologic evidence concerning the re-entry hypothesis for ectopic beats. *Amer. Heart J.* **72**: 60.
37. Hiraoka, M., and H. H. Hecht. 1971. Recovery from the prolonged cooling of cardiac Purkinje fibers. *Fed. Proc.* **30**: 667.
38. Repke, K. 1963. Metabolism of cardiac glycosides. *In* Proceedings of the First International Pharmacological Meeting, Stockholm, 1961. Vol. 3. *New Aspects of Cardiac Glycosides.* W. Wilbrandt, editor. Pergamon Press, Inc., Elmsford, N. Y.
39. Schwartz, A., J. C. Allen, and S. Harigaya. 1969. Possible involvement of cardiac Na^+, K^+-adenosine triphosphatase in the mechanism of action of cardiac glycosides. *J. Pharmacol. Exp. Ther.* **168**: 31.
40. Akera, T., F. S. Larsen, and T. M. Brody. 1970. Correction of cardiac sodium- and potassium-activated adenosine triphosphatase activity with ouabain-induced inotropic stimulation. *J. Pharmacol. Exp. Ther.* **173**: 145.
41. Repke, K. 1965. Effects of digitalis on membrane adenosine triphosphatase of cardiac muscle. *In* Proceedings of the Second International Pharmacological Meeting, Prague, 1963. Vol. 4. *Drugs and Enzymes.* B. B. Brodie and J. R. Gillette, editors. Pergamon Press, Oxford. 65.
42. Okita, G. T. 1967. Binding of cardiac glycosides. *In* Factors Influencing Myocardial Contractility. R. D. Tanz, F. Kavaler, and J. Roberts, editors. Academic Press Inc. New York. 549.
43. Glynn, I. M. 1964. The action of cardiac glycosides on ion movements. *Pharmacol. Rev.* **16**: 381.
44. Langer, G. A. 1968. Ion fluxes in cardiac excitation and contraction and their relation to myocardial contractility. *Physiol. Rev.* **48**: 708.