The Effect of Digitalis (Cedilanid) on the Mechanical and Electrical Activity of Extracted and Nonextracted Heart Muscle Preparations

By H. Stutz, M.D., E. Feigelson, M.S., J. Emerson, Ph.D., and R. J. Bing, M.D.

The purpose of this investigation was to study the effect of Cedilanid on the mechanical and electrical activity of extracted and nonextracted heart muscle strips of dogs. The glycoside did not influence the work, the speed of contraction, or the length–resting tension relationship of extracted heart muscle. It produced changes in membrane action potentials, but none in membrane resting potentials. The problem of whether digitalis is bound to the membrane or the cell protein was investigated by temporary destruction of the cell membrane by water. The effects of cardiac glycosides on contractile proteins and on membrane activity are probably inseparable; they act through changes in ionic gradient between muscle cells and the surrounding medium.

A DIRECT action of cardiac glycosides on the heart muscle has been demonstrated by a series of investigators. Cattell and Gold, by using an electrically driven papillary muscle of the cat, showed that the fatigued muscle regained its force of contraction after addition of digitalis to the solution in which the muscle was suspended. Similar results were obtained by Kabat and Visscher and by Feigen and colleagues. St. George, Friedman, and Ishida found that digitoxin failed to combine with the mitochondrial components of the cell, but was found within the components of the cell containing actin, myosin and adenosine triphosphate, suggesting that digitoxin may exert its effect directly upon the contractile elements of the cardiac cell.

The effect of digitalis on contractile proteins of heart muscle was further studied. Robb and Mallov found that ouabain caused an actomyosin thread to undergo greater and more rapid shortening. Bowen was of the opinion that digoxin produced the effect on muscle by altering the contractile proteins or their union with adenosine triphosphate. Horvath and colleagues came to the conclusion that the basic effect of ouabain may be on polymerization of actin. Snellman found that cardiac glycosides have a direct influence on the contractile proteins, possibly reversing the binding of ions in the contractile system and thus influencing the ionic balance in the environment of the muscle fiber. According to this author, the action of digitalis is directed against a substance which in different states is conjugated to protein and can show adenosine triphosphatase property.

The effect of digitalis preparations on the extracted heart muscle preparation have so far not been studied although general properties of such preparations have been previously described. It could be shown that heart muscle strips extracted in glycerol and water do not respond to usual electrical and chemical stimulations but contract only after addition of adenosine triphosphate in a proper ionic medium. It appeared from the work of Szent-Györgyi that actin and myosin in this preparation are associated to form actomyosin and that relaxation, if it occurs, is probably not commensurate with a disassociation of actomyosin.

Besides acting on contractile proteins, cardiac glycosides are known to exert an effect on the membrane action potentials of heart muscle. Woodbury and Hecht, using microelectrodes inserted into individual muscle fibers of frog's heart, found that digitoxin lengthens and then shortens membrane action potentials.
Toxic doses brought about extreme shortening of repolarization. The duration of depolarization was not affected. However, the amplitude of the depolarization diminished with large doses of the glycoside. Resting potentials were not changed.

A similarity of the effect of strophanthin to that of sodium lack on the intact heart was found by Daly and Clark. A decrease in the sodium concentration in the medium in which the muscle fibers were soaked resulted in a loss of overshoot. Furthermore, the action potential became shorter with no change in resting potential. Replacement of sodium chloride with isosmotic saccharose resulted in similar changes.

It is the purpose of this study to reinvestigate the effect of cardiac glycosides on the contractile proteins of heart muscle, using the washed heart muscle preparation previously described. Since, in the course of this investigation, it became apparent that cardiac glycosides had no effect on this preparation, the studies were extended to include experiments on membrane activity of isolated extracted and unextracted heart muscle strips of the dog. Thus, it was hoped to obtain some information on the location of action of the glycoside.

**MATERIAL AND METHODS**

*Experiments on Extracted Heart Muscle.* Muscle strips from hearts of 21 dogs were prepared according to the method previously described. Twelve of these animals had been digitalized for five days by daily intramuscular injections of 0.5 cc. (0.1 mg.) of Cedilanid. Cedilanid was also added to the glycerol water mixture and the fluid in which the muscle contracted to make a dilution of 1/30,000. Nine animals, serving as controls, received no digitalis and muscle strips from their hearts were extracted and contracted in solutions containing no glycoside.

Three sets of experiments were performed. In the first series, the isotonic contractions of 180 extracted heart muscle strips were recorded following addition of 0.8 per cent adenosine triphosphate and the speed of contraction and the relation of the work performance to rising tension were investigated as previously described.

In the second series, the effect of Cedilanid on the length-resting tension relationship of extracted heart muscle was studied. A strain gage manometer (Statham) calibrated with loads varying from 0.5 to 2 Gm. was used to measure the changes in tension resulting from alterations in length. Tension was expressed in milligrams per square millimeter and was plotted against percentage change in muscle length. The preparation of digitalized and nondigitalized muscle strips for these experiments was identical to that already described.

*Effect on Action Potentials.* The third series of experiments dealt with the effect of the glycoside on the membrane activity (membrane action and resting potential) of extracted and nonextracted heart muscle. Hearts from 31 dogs anesthetized with Nembutal were placed into normal tyrode solution kept at 37 C. and a pH of 7.3. Muscle strips of 0.5 to 1 mm. thickness and of 2 to 3 cm. in length were dissected from the left ventricle. The bundles were tied on wooden sticks and clamped into a tray through which the test solution circulated (fig. 1). The fibers were stimulated with a Grass Stimulator through a Grass Isolation unit. The muscles were excited through two thin stainless steel wires attached directly to the muscle bundle—about 0.5 mm. apart. Stimuli from 0.1 to 1.5 milliamperes were used at a frequency of 1 per second and a duration of 6 milliseconds.

The membrane action potentials were led off by a microelectrode balanced against an indifferent stainless steel electrode placed in the bath at a distance from 1 to 2 cm. from the muscle and attached to one tube of a preamplifier. The exploring electrode was a glass microelectrode filled with 3 M potassium chloride. A thin stainless steel wire connected this microelectrode with the second tube of the preamplifier. This tube was placed in close proximity to the microelectrode.

Membrane action potentials and resting poten-
Fig. 2. Isotonic contraction of extracted digitalized and nondigitalized heart muscle after addition of 0.8 per cent adenosine triphosphate. No difference between the digitalis and the control group is noticeable.

The microelectrodes were prepared according to the method of Ling and Gerard. Three molar potassium chloride was employed to fill the electrodes. This was accomplished by boiling the electrodes in this solution. After rapid cooling, the electrodes were placed in vacuum to assure better filling. Only microelectrodes with a resistance of between 20 and 100 megohms were used. The outside diameter of the electrode tip varied from 0.5 to 1 micron. One factor causing a bias in the measurement in the membranes resting and action potentials is the junction potential between the needle electrode filled with potassium chloride and the cytoplasm of the cell. Since the exact composition of the cytoplasm is still unknown, detailed calculations for the purpose of correction are impossible. Curtis and Cole and Woodbury and Hecht obtained an estimated value of this junction potential of between 6 and 3 mv. respectively, with a sign such that the observed action potentials would be decreased. Since the observed voltages are many times the junction potential, this correction is negligible.

Tyrode's solution was used to which glucose was added to make a concentration of 100 mg. per 100 cc. A pH of from 7.3 to 7.4 with a carbon dioxide pressure of 44 mm. Hg was maintained in this solution by addition of sodium carbonate, by introducing a gas mixture of 90 per cent oxygen and 10 per cent carbon dioxide, and by maintaining a temperature of 38 C. In some of the experiments the muscle fibers were extracted in distilled water of 38 C. exposed to the same amount of oxygen and carbon dioxide. The pH of the water varied from 5.6 to 6.6.

Cedilanid in the following dilutions was used: $1:5 \times 10^4; 1:5 \times 10^5; 1:6.3 \times 10^5$ and $1:7.2 \times 10^5$. The solutions were prepared and stored in 2000 ml. jars at a temperature from 38 to 39 C. The insertion of the microelectrodes was carried out with the aid of a specially built microactuator under microscopic visualization.*

RESULTS

Relation between Work Load and Initial Tension and between Initial Tension and Speed of Contraction

Previous work had shown that the work of the extracted muscle strip increased with rising

* Manufactured by Electrical Research Corporation, Atlanta, Ga.
tension up to a maximal value and decreased as this tension was exceeded. Prolonged periods of stretch, leading to an increase in initial length resulted in greater work performance of the extracted muscle. Figure 2 shows that as in previous published experiments, the force of contraction increased with rising loads up to an optimal value and decreased when the tension became excessive. It may also be seen that Cedilanid had no effect on the work-tension relationship of extracted heart muscle strip.

Since the degree of shortening of extracted heart muscle was found to be directly proportional to the logarithm of time, the slope of the contraction curve can be expressed as a function of the logarithm of time and the degree of shortening. Thus, the slope is directly proportional to the speed of contraction. Figure 3 illustrates that, as in previous published work, the speed of contraction, expressed by its logarithmic slope decreased with rising tension.

It also may be seen that the speed of contraction of digitalized extracted heart muscle appeared to be identical with that of nondigitalized muscle strips.

**Effect of Cedilanid on Length–Resting Tension Relationship**

It has been maintained that the effect of digitalis is on the heart muscle directly expressing itself in changes in initial fiber length and/or initial tension. If this were the case, the cardiac glycoside could conceivably influence the length–resting tension relationship of extracted heart muscle by a relatively greater tension per increase in length. Figure 4 illustrates that Cedilanid had no effect on the relationship between length and resting tension of extracted heart muscle. These results are in agreement with the findings of Kabot and Visscher who found that digitalis did not alter the diastolic tone of fresh heart muscle.
Effect of Cedilanid on Membrane Activity of the Extracted and Nonextracted Heart Muscle

A. Effect of Cedilanid on Muscle Suspended in Tyrode. Muscle strips were suspended in Tyrode’s solution to which Cedilanid in varied concentrations had been added. Figure 5a illustrates a normal action potential. The effects of Cedilanid are illustrated in figures 5b and 5c. The first sign of glycoside activity consisted in spontaneous contractions, succeeding the contractions resulting from electrical stimulation. These “after-contractions” were of shorter duration with steeper and shorter repolarizations. The changes in action potentials themselves were similar to those previously described. The first effect consisted in a loss of overshoot; following this, the amplitude of depolarization diminished. The first phase of repolarization became steeper and of shorter duration, while phases 2 and 3 became flatter. Thus, the whole action potential shortened. Signs of extreme toxicity consisted in irregular spontaneous contractions, fibrillation and arrest. The speed with which these changes appeared seemed to depend on the concentration of Cedilanid, with higher concentrations resulting in a more rapid appearance of changes described. Similar to the results obtained by Woodbury and Hecht, digitalis had no effect on the membrane resting potential.

In order to investigate the binding of Cedilanid to heart muscle, fibers which had been bathed in Tyrode’s solution containing Cedilanid and which had exhibited glycoside effects, were placed in the Cedilanid-free Tyrode’s solution. The alterations in action potential persisted and often progressed until the muscle failed to react.

B. Effect of Water Extraction on Digitalized Heart Muscle. In 13 experiments the influence

Fig. 4. Effect of Cedilanid on the length–resting tension relationship of digitalized and non-digitalized extracted heart muscle strip. No difference is noticeable. The points represent average values of tension developed with percentage changes in length.
EFFECT OF CEDILANID ON HEART PREPARATIONS

of water extraction on the action potential of digitalized muscle strips was studied. The muscle was bathed in Tyrode's solution containing Cedilanid until typical alterations appeared. The fibers were then extracted in water until all mechanical and electrical activity had ceased. This usually occurred after two to six minutes. Following this, the water was replaced with Cedilanid-free Tyrode's solution in order to restore muscle activity. Figure 6 shows that the restored action potential had maintained all the features of digitalis effect.

C. Response of the Washed Heart Muscle to Digitalis. Eight experiments were performed dealing with the action of the glycosides on washed heart muscle strips in which the membrane activity had been abolished. The fibers were suspended in water until the action potentials had disappeared. This occurred usually in about three to six minutes. Following this, the muscles were bathed in Tyrode's solution containing Cedilanid. Electrical activity usually appeared after 8 to 15 minutes' contact with this solution. The action potential showed from the beginning the pattern of digitalis effect.

D. The Effect of Water and Glycerol Extraction on Electrical Activity. These experiments represent controls, since they did not involve the action of Cedilanid. Heart muscle fibers were suspended in water with or without glyce-
FIG. 6. Membrane action potential obtained from muscle immersed in Cedilanid-free Tyrode's solution. The muscle had been previously digitalized in Tyrode's solution containing Cedilanid, followed by water extraction for six minutes. The restored action potential had maintained all the features of digitalis effect.

FIG. 7. Effect of water and glycerol extraction on membrane action potential of heart muscle. There was increase in latent period to approximately 100 milliseconds. The amplitude of depolarization diminished, and the duration of repolarization widened.

FIG. 8. Water extraction leads to an average fall of 40 to 45 per cent in membrane resting potential. This occurs only after resting membrane potentials have ceased during extraction shortening to 150 to 175 milliseconds). Furthermore, the period from the stimulation artefact to the commencement of action potential (the latent period) increased. Following this, the electrical response disappeared abruptly after two to seven minutes. It was found that the thicker strips maintained electrical activity longer than thinner ones. During extraction, the membrane resting potential dropped an average of 40 to 45 per cent (about 35 mv.) (fig. 8). Extraction of the muscle in water and glycerol for periods exceeding seven minutes completely abolished electrical activity. However, when the muscle was immersed in Tyrode's solution after 2 to 10 minutes of water extraction, action potentials reappeared. There occurred first a rise in resting potential, then action potentials of lesser amplitude reappeared. After two or three muscle contractions the action potential had regained its former configuration and amplitude.

DISCUSSION

The results demonstrate that Cedilanid has no effect on the work–tension relationship, the speed of contraction and the length–resting tension relationship of extracted heart muscle preparations (figs. 2, 3 and 4). If it is assumed that the extracted heart muscle strip represents...
essentially a model of the contractile proteins in which normal excitatory stimuli for contraction are lost, one might interpret these findings as evidence against an interaction of the glycoside with the contractile matter of heart muscle. This is in contrast with results reached by Robb and Mallow; these investigators demonstrated that when ouabain was added to an aliquot of actomyosin solution, prior to surface film formation and compression of this film into actomyosin threads, the threads shortened to a greater extent and at a more rapid rate than threads containing no glycoside. The discrepancy between our findings and those of Robb may be due to several factors. Robb's fibers represent relatively pure actomyosin; the washed heart muscle strips contain sarcolemma and the histologic structure of the fiber is well preserved. In Robb's experiments, the thread was in intimate contact with the glycoside since ouabain was added directly to the actomyosin solution. In the experiments reported in this paper, diffusion of the glycoside into the muscle may have been a limiting factor. Furthermore, periods of extraction in water and glycerol for several days may have broken the link between the glycoside and the contractile substances. Snellman has stated that cardiac glycosides act only when the contractile substance is coupled with protein. Prolonged water extraction may have destroyed this combination.

The effect of Cedilanid on the membrane action potential of heart muscle strips soaked in Tyrode's solution consists in loss of overshoot, a diminution in the amplitude of depolarization and shortening of repolarization. The latter is primarily the result of diminution in length of the first phase of repolarization. Eventually the whole shape of repolarization steepens, (fig. 5b and c) and irregular spontaneous contractions appear, followed by fibrillation and arrest. The results are similar to those reported by others using single ventricular fibers. Woodbury and Hecht explain the differential effect of the glycoside on the individual phases of repolarization by postulating a different type of interference with the metabolic process occurring during recovery. Similar to the results of Woodbury and Hecht, Cedilanid produced no change in membrane resting potential.

The strong binding power of heart muscle to the glycoside is illustrated in experiments in which it is shown that membrane action potentials of fibers which had been bathed in Tyrode's solution containing Cedilanid maintained their digitalis effect when placed in Cedilanid-free Tyrode's solution. Theoretically, the glycoside could have remained attached to the membrane or it could have penetrated the interior of the cell combining with the contractile proteins. The experiments in which the action potential of the digitalized muscle were studied following extraction in water were designed to elucidate this question.

When muscles in Tyrode's solution containing Cedilanid are extracted in water for two to six minutes, all mechanical and electrical activity ceases. Action potentials demonstrating digitalis effect reappear, however, when the muscle is brought into contact with Cedilanid-free Tyrode's solution (fig. 6). If it is assumed that water extraction destroys the membrane temporarily, then the glycosides must have become fixed to the interior of the cell during soaking in water, exerting its influence immediately upon restoration of the membrane in Tyrode's solution. On the other hand, the glycoside may have been bound to the membrane itself, which may have only temporarily lost its ability of depolarization and repolarization, presumably through changes in permeability to sodium and potassium.

When membrane activity is initially abolished by soaking the muscle strips in water for three to six minutes and the fibers are then exposed to Tyrode's solution containing Cedilanid, the reappearing action potentials bear all the marks of glycoside activity. Here too, it is difficult to decide whether or not Cedilanid had been bound to the contractile proteins. The fact that the membrane action potentials reappeared at all makes it unlikely that the membrane had been destroyed during the process of extraction. It is obvious that prolonged extraction results in disappearance of membrane action potentials regardless of whether or not the strip has been digitalized.
Extraction of muscle strips in water alone produces changes which resemble those initiated by Cedilanid (fig. 7). There gradually occurs incomplete depolarization and a decrease in slope of repolarization. There is also an increase in duration of the latent period until all electrical activity ceases abruptly. In contrast to results obtained after water extraction of digitalized muscle, the resting membrane potential declines during extraction, a finding which is in agreement with that obtained by Tobias\textsuperscript{17} (fig. 8).

The similarity between the effect of water extraction and that of Cedilanid is possibly the result of electrolyte changes which occur under both conditions. Extraction of the fiber leads, as Tobias\textsuperscript{17} has shown, to loss of potassium, sodium and other substances. Daly and Clark\textsuperscript{12} found a similarity between the effect of strophanthin and that of sodium lack. Cardiac glycosides are known to alter the electrolyte equilibrium between heart muscle cell and plasmas.\textsuperscript{18} A current view holds that depolarization of the membrane first causes an increase of sodium permeability, this phase changing after a brief delay into one of increased potassium permeability. Apparently, active secretion of sodium ions is continuously at work in the fiber to maintain a low internal sodium concentration against relatively slight continual leakage.\textsuperscript{19} Digitalis may possibly alter this exchange.

The finding that water extraction reduces but does not abolish resting membrane potential has been explained by Tobias\textsuperscript{17} as evidence of asymmetry in the cell, possibly in the protein mass which can determine the orientation of the potential in the normal cell and may be responsible for a multiple origin of the electromotive force seen in the normal cell. Whether glycosides affect the source of this electromotive force is still a moot point.

From the results reported in this paper and those published in the literature, it appears that the effect of cardiac glycosides on contractile proteins and its effect on membrane activity are probably inseparable and act through a common denominator. This may well consist of a change in the ionic gradient between muscle cell and the surrounding medium. Ions play a role in every phase of muscular contraction. The intracellular ionic atmosphere determines the changes, the balance of forces and the behavior of actin and myosin toward each other. Equally, electrical manifestations during excitation are the expression of ionic changes.\textsuperscript{20} The membrane not only conducts the excitation impulse, it also regulates, through selective permeability, the ion concentration within the cell which decides the behavior of the contractile proteins. For this reason, membrane activity and physical-chemical pattern of contractile proteins are probably inseparable.

**Summary**

The effect of Cedilanid on the mechanical and electrical activity of extracted and non-extracted heart muscle of dogs was studied. Microelectrodes were used as exploring electrodes.

Cedilanid did not influence the work, the speed of contraction or the length–resting tension relationship of extracted heart muscle. It caused changes in membrane action potential of heart muscle strips consisting in loss of overshoot, steepening of the first phase and flattening of phases 2 and 3 of repolarization. The glycoside had no effect on membrane resting potential.

Fibers which had been bathed in Tyrode's solution containing Cedilanid maintained their digitalis effect when placed in Cedilanid-free Tyrode's solution. Digitalization of the fibers in Tyrode's solution followed by short periods of water extraction resulted in cessation of all electrical activity. Cedilanid-free Tyrode's solution restored membrane action potentials demonstrating digitalis effect.

Short periods of extraction in water followed by exposure to Tyrode's solution containing Cedilanid resulted in reappearance of action potentials with characteristic digitalis pattern. Extraction of muscle strips in water alone produced, first, incomplete depolarization, then a decrease in shape of repolarization and increase in latent period. Following this, all electrical activity ceased. In some respects, these changes resembled those produced by the glycoside. In contrast to Cedilanid, water ex-
traction reduced the resting membrane potential.

It appears that the effects of cardiac glycosides on contractile proteins and on membrane activity are inseparable and act through changes in ionic gradient between the muscle cell and the surrounding medium.

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