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Ouabain-Induced Mechanical Toxicity: Aberrations in Left Ventricular Function, Calcium Concentration, and Ultrastructure (41805)

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Abstract. The isolated rabbit heart was employed to determine if the myocardial mechanical dysfunction (mechanical toxicity) produced by digitalis was accompanied by ultrastructural lesions and an excessive accumulation of calcium by the cardiac cell. Ouabain (1.2 or 2.4 μM) produced a transient increase and then a continuous decrease in the systolic pressure development by the isovolumic left ventricle. The end-diastolic pressure increased progressively during the time that the systolic pressure was diminishing. The ultrastructural abnormalities that were observed in the ouabain-treated hearts included swollen mitochondria, hypercontracted sarcomeres and, occasionally, myofibrillar disruption. The left ventricular cellular calcium concentration was increased ($P < 0.05$) in these mechanically toxic hearts and this increase correlated significantly ($r = 0.65$, $P < 0.05$) with the magnitude of the increase in end-diastolic pressure. Furthermore, a reduction in the perfusate calcium concentration did not attenuate the ouabain-stimulated uptake of calcium. The data suggest that the impaired mechanical performance and the ultrastructural aberrations that may develop in the digitalis-treated heart are related to an excessive uptake of calcium by the left ventricle.

Electrical cardiotoxicity (arrythmias) is one of the principal manifestations of an overdose of digitalis. The possibility of serious ventricular arrhythmias during digitalis treatment is recognized widely, and the cause of the arrythmias is established (1, 2). Mechanical toxicity (contractile dysfunction) is another major manifestation of a digitalis overdose (3–7). Our preliminary experiments (8) demonstrated an enhancement and then a depression in left ventricular function when the coronary-perfused rabbit heart was exposed to larger doses of ouabain. The cause of the cardiac mechanical dysfunction produced by digitalis is unknown. It has been postulated by Vassalle and Lin (7) that this contractile deterioration is due to an excessive uptake of calcium by the cardiac cell. Several groups of investigators (3–5) have examined the effect of 15–30 min of treatment with toxic doses of digitalis on myocardial calcium concentration, but the results were variable.

In our study, we sought to demonstrate that a dose of digitalis which causes mechanical toxicity in the rabbit heart results in an elevation in myocardial calcium concentration. If an increase in calcium concentration was found, we proposed to determine: (a) if a decrease in the extracellular calcium concentration reduces the severity of the toxicity and attenuates the uptake of calcium by the myocardial cell, and (b) if ultrastructural lesions, similar to those produced by other interventions that increase intracellular calcium, are present in the intoxicated cardiac tissue. It is well-known that when the cardiac cell accumulates a large quantity of calcium, its mechanical performance is impaired (9–15), and sometimes ultrastructural lesions develop (13–16). The intact rat heart accumulated calcium and developed lesions and functional deficits after the subcutaneous injection of a large dose of isoproterenol (13, 15). Similar changes occurred in the isolated rat heart when normal perfusion conditions were restored after a period of calcium-free perfusion (9, 14) and also during potassium-free perfusion (16).

Materials and Methods. *Experimental preparation.* The white, New Zealand rabbit (2–3 kg) was thoracotomized after anesthetization with intravenous sodium pentobarbital (40 mg/kg) and the onset of positive-

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pressure ventilation with 100% O_2. Heparin (1000 U, iv) was administered, the aorta was cannulated retrogradely for coronary artery perfusion, and the heart was removed from the thoracic cavity. The bicarbonate-buffered perfusate, which was gased with 95% O_2–5% CO_2, had the following composition (mmole/liter): NaCl 89, KCl 5, $MgSO_4$ 1, $NaHCO_3$ 24, Na_2HPO_4 1, $CaCl_2$ 2.0 or 0.5, dextrose 10, and sodium acetate 20. Immediately following isolation of the heart, the interventricular septum was crushed to dissociate the ventricular from the atrial contractions and the ventricles were paced at 90 beats per min by square pulses of 4-msec duration and 4-V intensity (Grass, S44). A fluid-filled balloon was passed into the left ventricle through the mitral valve as described previously (17, 18). The balloon volume was adjusted so that the end-diastolic pressure was 10 mm Hg, and thereafter the left ventricular isovolumic pressure development (Gould–Statham, P23ID) was recorded continuously (Gould polygraph, 2600). Perfusate flow rate of 35 ml/min and temperature of 30°C were maintained constant throughout each experiment.

Since one of our primary aims was to determine if there was a correlation between the ouabain-induced mechanical dysfunction and the cellular calcium concentration, it was desirable to evaluate the changes in left ventricular function in an arrhythmia-free preparation. Propranolol (1.5 mg/liter) was added to the ouabain-containing perfusates to abolish the electrical abnormalities that are characteristically associated with digitalis toxicity. This concentration of propranolol (1.5 mg/liter) also was present in the control perfusate.

Experimental procedures. Each heart was equilibrated for 90 min with control perfusate prior to the onset of 60 min of perfusion with 1.2 (N = 13) or 2.4 (N = 6) μM ouabain-containing solution. In most experiments, the perfusate calcium concentration was 2.0 mM, but was reduced to 0.5 mM in four experiments. Some degree of mechanical toxicity was displayed by each preparation at the end of ouabain treatment. Control hearts (N = 9) were subjected to the 90-min equilibration procedure and an additional 60 min of ouabain-free perfusion.

When the heart was removed from the perfusion circuit, the atria and the right ventricle were trimmed from the heart, and the left ventricle was frozen in liquid nitrogen and stored (−90°C) for later analysis of its water and calcium contents. The left ventricular water content was calculated from the difference between the wet and dry weights of a left ventricular tissue sample. The wet tissue samples had masses of 600–800 mg and were dried to a constant weight at 80°C. Calcium concentration was measured in each of three tissue samples from each heart. One tissue sample was taken from the basal, one from the middle, and one from the apical region of the left ventricle. The calcium was extracted from each sample (300–400 mg) with equal parts of glacial acetic acid and trichloroacetic acid as described by Sparrow and Johnstone (19), and the calcium concentration in the tissue extract was measured with an atomic absorption spectrophotometer (Varian, AA-375). The left ventricular tissue calcium concentration was considered to be the average of the three tissue sample concentrations. The cellular calcium concentration was calculated as the difference between the tissue concentration and the extracellular fluid concentration. It was assumed that the perfusate and the extracellular fluid calcium concentrations were identical. The volume of the extracellular space was considered to be 43.9% of the tissue water content and was assumed to remain constant during the ouabain perfusion. The constant, 0.439, represents the ratio of extracellular volume to tissue water content and was determined from the diffusable sucrose space measured in separate experiments under the same perfusion conditions. This value agrees very closely with the extracellular volume:tissue water ratio of 0.44 reported by Page and Polimeni (20) for isolated, perfused rat hearts.

Two ouabain-treated (2.4 μM) hearts and one control heart were fixed for electron microscopy by perfusing for 5 min with 2% paraformaldehyde and 1% glutaraldehyde in physiological saline. The pH of the fixative was adjusted to 7.4 with NaOH. Blocks of tissue (less than 1 mm^3), taken from the basal, middle, and apical regions of the left ventricular free wall and septum, were placed in the fixative for an additional 90 min. The tissue was washed subsequently in physiological saline and postfixed with 1% OsO_4, dehydrated in ethanol, and embedded in Epon according to

Luft (21). Gold sections were poststained with uranyl acetate (2%) and Reynolds' lead citrate (22). The sections were cut with a diamond knife on a Sorvall MT 5000 ultramicrotome and the tissue was examined with a JEOL 100-S transmission electron microscope. Sarcomere lengths were determined from electron photomicrographs. Calipers were employed and measurements from five consecutive sarcomeres were averaged.

Student's *t* test was used to determine if the calcium concentrations in the ouabain-treated hearts were significantly different from those in the control hearts. The values reported in the text and figures are means ± 1 SE.

Results. *Left ventricular mechanical function.* The increase in left ventricular contractility after onset of ouabain treatment was transient (Fig. 1). The peak inotropic effect was reached within 15 min and was followed by a continuous decline in mechanical performance. The mechanical dysfunction after onset of ouabain treatment also was characterized by a continuous increase in end-diastolic pressure (Fig. 2). The control preparations were stable as demonstrated by the constancy of both active pressure development (Fig. 1) and end-diastolic pressure (Fig. 2).

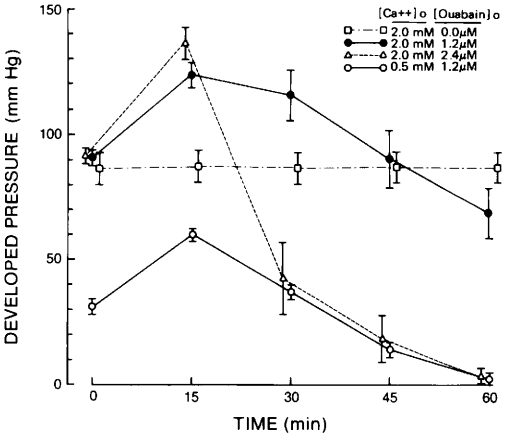

FIG. 1. Effect of ouabain on left ventricular function. Pre-ouabain values for developed pressure—the systolic pressure minus the diastolic pressure—are shown at time zero. Mechanical toxicity was evident with both 1.2- and 2.4-μM ouabain doses. At 60 min, pressure development was obliterated by the 2.4-μM ouabain dose in 2.0 mM calcium and also by the 1.2-μM ouabain dose in 0.5 mM calcium. Thus, the reduction in external calcium concentration aggravated rather than ameliorated the toxic actions of ouabain. The function of the control hearts did not change during the 60-min experimental period.

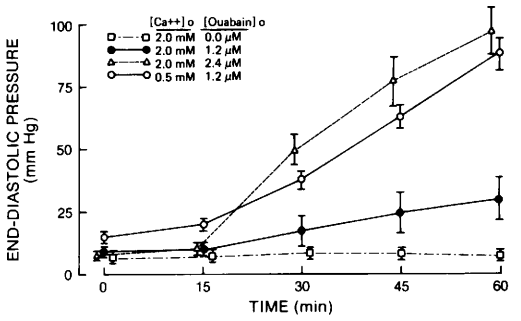

FIG. 2. Development of contracture during ouabain treatment. Hearts treated with 1.2 μM ouabain and 0.5 mM calcium developed the same degree of contracture as hearts treated with 2.4 μM ouabain and 2.0 mM calcium. The magnitude of the contracture was reduced when the ouabain dose was 1.2 μM and the calcium concentration was 2.0 mM. No change in end-diastolic pressure was observed in the control hearts.

The severity of the left ventricular failure was dependent on the ouabain dose and the calcium concentration. After the peak positive inotropic effect was reached in 2.0 mM calcium solution, there were smaller decrements in active pressure development and smaller increments in end-diastolic pressure with the 1.2-μM ouabain dose than there were with the 2.4-μM ouabain dose (Figs. 1 and 2). The 2.4-μM ouabain dose in the perfusate containing 2.0 mM calcium led to a complete loss of pressure-developing ability (Fig. 1). Pressure development also was completely dissipated when the perfusate calcium concentration was 0.5 mM and the ouabain dose was 1.2 μM (Fig. 1). Furthermore, the increases in end-diastolic pressure when the perfusate calcium concentration was 0.5 mM and the ouabain concentration was 1.2 μM were similar to those that occurred when the perfusate calcium concentration was 2.0 mM and the ouabain concentration was 2.4 μM (Fig. 2). Hence, the contracture which was induced by the 1.2 μM dose of ouabain was accentuated when the extracellular calcium concentration was reduced.

Left ventricular calcium concentration. Both tissue and cellular left ventricular calcium concentrations increased significantly compared to control when hearts were exposed to

the perfusate with either 1.2 or 2.4 μM ouabain (Fig. 3). The reduction in perfusate calcium concentration to 0.5 mM did not protect against this ouabain-stimulated uptake of calcium (Fig. 3). The increase in cellular calcium produced by treatment with 1.2 μM ouabain during 0.5 mM calcium perfusion was the same as the increase produced by treatment with 2.4 μM ouabain during 2.0 mM calcium perfusion (Fig. 3). The hearts exposed to 2.4 μM ouabain in 2.0 mM calcium and to 1.2 μM ouabain in 0.5 mM calcium were the most toxic (Figs. 1 and 2) and also contained the most calcium (Fig. 3). This observation was supported by data which showed a positive correlation ($r = 0.65$) between the cellular calcium concentration and the left ventricular end-diastolic pressure after 1 hr of ouabain treatment (Fig. 4). The tissue water contents which were used to derive the extracellular volumes and cellular calcium concentrations were (μl/g): 813 ± 3 when ouabain and calcium concentrations were 0.0 μM and 2.0 mM, respectively; 808 ± 3 when ouabain and calcium concentrations were 1.2 μM and 2.0 mM, respectively; 820 ± 3 when ouabain and calcium concentrations were 2.4 μM and 2.0 mM, respectively; and 840 ± 1 when ouabain and calcium concentrations were 1.2 μM and 0.5 mM, respectively. The calculated extracellular spaces ranged between 355 ± 1 and 370 ± 1 μl/g.

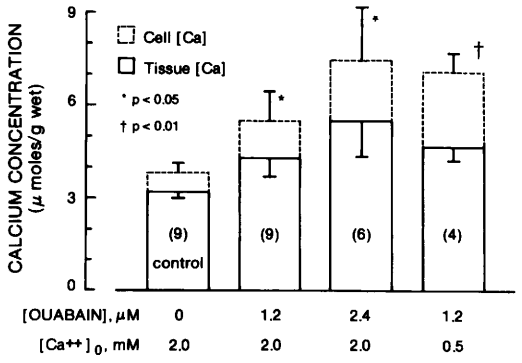

FIG. 3. Left ventricular calcium concentrations after ouabain treatment. Both doses of ouabain (1.2 and 2.4 μM) caused a significant increase in left ventricular calcium concentration. The reduction in calcium concentration to 0.5 mM did not retard the ouabain-stimulated uptake of calcium by the cardiac cell. The number of experiments per experimental group is shown in parentheses.

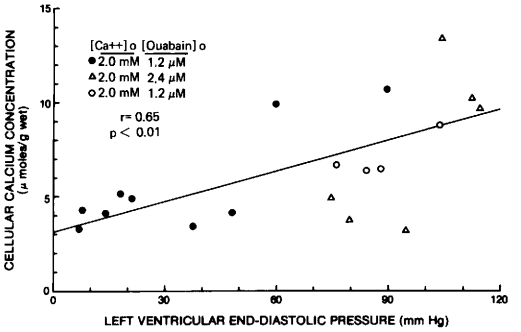

FIG. 4. Relationship between left ventricular calcium concentration and left ventricular end-diastolic pressure. The correlation between the cellular calcium concentration and the end-diastolic pressure ($r = 0.65$) was significant ($P < 0.01$).

Left ventricular ultrastructure. The structural integrity of the ventricular cell was disrupted to various degrees by ouabain treatment (Figs. 5 and 6). Hypercontracted sarcomeres were evident in most of the sections that were prepared from the ouabain-treated tissue. The average length of the sarcomere of the treated hearts was 1.5 μm (Fig. 6A) whereas the control heart had an average sarcomere length of 2.1 μm (Fig. 5). Swollen mitochondria and intracellular edema were apparent in almost all of the tissue sections from the ouabain-treated hearts (Fig. 6A). A few regions of the treated hearts were more severely deranged. In these regions there were wavy Z lines, a loss of continuity between parallel myofibrils, and disintegrated sarcomeres (Fig. 6B). There was no apparent relationship between the degree of tissue disruption and the site in the ventricular wall from which the specimen was taken.

Discussion. The concentrations of ouabain employed in this study were two- to fivefold greater than those used by other investigators to study the therapeutic actions of this drug (23, 24). These doses caused a transient increase and then an impairment in left ventricular mechanical performance of the isolated rabbit heart as well as an increase in tissue and cellular calcium. The increases in diastolic pressure correlated significantly with the increases in cell calcium. Verification of the contracture (increased end-diastolic pressure) was provided by electron photomicrographs which demonstrated the presence of

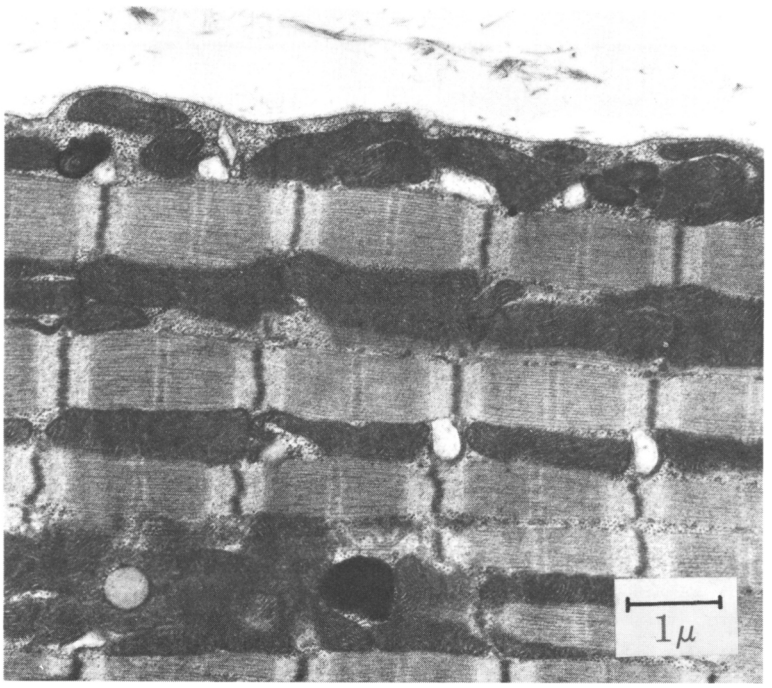

FIG. 5. Electron photomicrograph of left ventricular tissue from control rabbit heart (×12,500). The tissue of the control preparation had a normal appearance.

shortened sarcomeres in tissue sections from the ouabain-treated hearts. These findings support the hypothesis that the deterioration in cardiac function and structure associated with digitalis toxicity is due to an excessive uptake of calcium by the cardiac cell. The increased intracellular calcium appears to result from an altered sarcolemmal sodium–calcium exchange rate (25) secondary to the well-known ouabain-induced depression of the sarcolemmal Na^+, K^+-ATPase (26) and the subsequent increase in the intracellular sodium activity (27). It is unsettled as to whether an increase in calcium influx or a decrease in calcium efflux occurs when the intracellular sodium concentration increases (28).

Changes in cardiac structure and function similar to those we observed have been induced by other interventions that enhanced the flux of calcium into the cardiac cell (11, 13–15). The subcutaneous injection into the rat of a high dose of isoproterenol not only altered the structure, function, and calcium concentration of the heart (13, 15), but also produced a reduction in its content of adenosine triphosphate and creatinine phosphate (13). The depletion of high-energy phosphate stores, secondary to the flooding of calcium into the cardiac cell, may be the factor which triggers the structural deterioration of the myocardium of the isoproterenol-treated heart (13, 15) and also the ouabain-treated heart.

Data from previous studies of changes in tissue calcium concentration after exposure to toxic concentrations of cardiac glycosides lack harmony (3–5). Ouabain treatment produced an increase (3) or no change (4) in the calcium concentration of the guinea-pig atrium, and no change in the calcium concentration of the rabbit left ventricle (3), even though there was a decrease in contractile force and the development of contracture in each of these tissues. In contrast, the myocardial calcium concentration of the guinea-pig atrium decreased during the therapeutic phase and then increased toward the control level during the toxic phase of k-strophanthin or digitoxigenin action (5). The fact that the tissue calcium concentration did not increase in all preparations during toxic-dose treatment with digitalis does not refute our conclusions. The treatment times of these investigators (3–5)

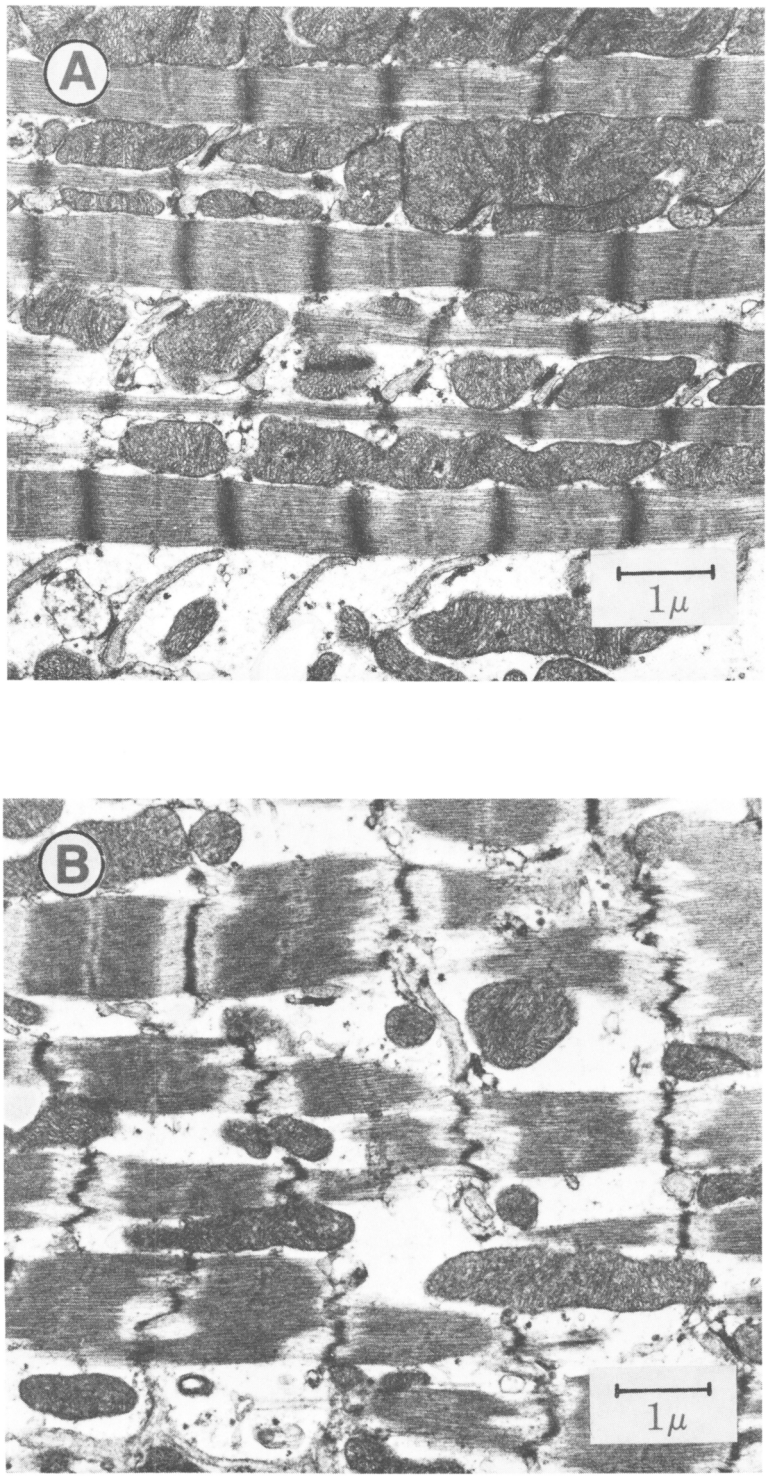

FIG. 6. Structure of left ventricular tissue after 2.4-μM ouabain treatment. Although all regions of the left ventricle were damaged after exposure to ouabain, some regions appeared to be more severely affected than others (compare B with A). ×12,500.

were substantially less than the 60-min treatment period of our study. Measurable increases in tissue calcium concentration may not have occurred during these shorter exposure times.

The ouabain-induced toxicity in our preparation was not prevented, nor was its rate of onset delayed, by reducing the perfusate calcium concentration from 2.0 m*M* to 0.5 m*M*. On the contrary, the increases in end-diastolic pressure produced by 1.2 μM ouabain in the presence of 0.5 m*M* calcium were the same as those produced by 2.4 μM ouabain in the presence of 2.0 m*M* calcium. Not only did the contracture develop to the same extent in each of these two groups of hearts, but the ventricular calcium concentration also increased to the same level. Myocardial tissue, therefore, is more vulnerable to the toxic actions of digitalis when the extracellular calcium concentration is reduced. Two plausible, but speculative, explanations could account for this apparent paradox. The greater accumulation of calcium by the cardiac cell in the presence of ouabain and decreased calcium may result from the presence of more ouabain at the sarcolemma. This possibility is based on the observation that the uptake of ouabain by the myocardial cell is inversely related to the external calcium concentration (29). Alternatively, the digitalis-induced increase in calcium influx may be coupled to a reduced rate of calcium efflux. The efflux of calcium from the cardiac cell has been shown to diminish when the external calcium concentration is decreased (30).

An impairment in myocardial function and an increase in cellular calcium concentration have been shown to occur after exposure of cardiac muscle to hypoxic conditions (12). Perfusion with an erythrocyte-free solution may have led indirectly to an accumulation of calcium by the ouabain-potentiated heart because the oxygen delivery in relation to metabolism was insufficient. However, this seems unlikely because the hearts that were treated with ouabain when perfusate calcium concentration was 0.5 m*M* generated peak inotropic pressures that were 25 mm Hg less than those developed by the control hearts. If anything, the oxygen demand of the ouabain-treated hearts during the 0.5-m*M* calcium perfusion was reduced compared to control. Since the control hearts had greater oxygen requirements but contained significantly less calcium than the hearts treated with ouabain and reduced calcium, hypoxia may be ruled out as the likely stimulus for calcium accumulation.

We determined the significance of the relationship between cell calcium concentration and end-diastolic pressure, rather than between total tissue calcium concentration and end-diastolic pressure, because two different extracellular calcium concentrations (0.5 m*M* and 2.0 m*M*) were employed. An alteration in extracellular calcium concentration produces a change in total tissue calcium concentration which does not necessarily reflect a change in cell calcium concentration. The assumption in our calculations of the cell calcium concentrations was that the extracellular volume was a fixed fraction of the total tissue water. If the extracellular volume changed in the toxic hearts, which is possible since fluid did accumulate in the hearts treated with 1.2 μM ouabain in the presence of 0.5 m*M* calcium, the intracellular calcium concentration may have been overestimated or underestimated relative to control. Our concern was that the fractional volume of the extracellular space of the digitalized hearts may have decreased compared with control. If this were the case, the extracellular space of the treated hearts would have been overestimated and an artifactually high intracellular calcium concentration calculated. In order for the extracellular volume to have been overestimated, however, the additional water would have had to distribute preferentially to the intracellular compartment. Since mean tissue hydrostatic pressure would be expected to be reduced in asystolic hearts, the accumulation of water by the ouabain-treated heart probably occurred because there was an increase in capillary filtration due to a shift in Starling forces. The shift of isosmotic fluid into the extracellular space from the capillary would not create an osmolality gradient across the cell membrane of the myocyte, and thus, the forces for the movement of additional water into the cell would remain negligible or nonexistent. In all likelihood, most of the additional water probably remained in the interstitial compartment. Therefore an error, if one exists, is likely to be in the conservative direction. In any event,

in all experimental groups and also in control hearts, the tissue calcium concentration was greater than the perfusate calcium concentration. Thus, an increase in tissue calcium concentration in the digitalized hearts could not have occurred unless the cell accumulated calcium.

Propranolol was incorporated into the perfusion solutions in order to suppress the arrhythmic activity and the accompanying erratic contractions that occur with higher-dose digitalis treatment. The correlation between left ventricular function and calcium concentration would have been impossible had the arrhythmias not been controlled. Also, since the flux of calcium into the cell is beat dependent, the arrhythmic bursts might have altered the intracellular calcium concentration. The question arises as to whether or not the calcium sequestration by the cardiac cell would have occurred in the absence of propranolol. Since propranolol depresses myocardial contractility by blocking β_1-receptor activity and retarding the flux of calcium into the cell, we conclude that the ouabain-stimulated increase in tissue calcium probably was antagonized, and not potentiated, by the propranolol.

The toxic manifestations that are known to occur in some humans who are treated with digitalis may involve a mechanical component. The cardiac status of some patients is worsened by digitalis treatment (31). There is the possibility that this deterioration in myocardial mechanical performance is related, in part, to an excess accumulation of calcium within the cardiac cell.

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