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Effects of Cadmium on Contractility and Calcium Concentration in Isolated Heart Muscle[1] (41378)

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Abstract. The isometrically arranged kitten heart papillary muscle was used to study the depressant actions of cadmium ion (Cd^{2+}) on heart muscle function. Although the Cd^{2+}-induced decreases in papillary muscle tension development were dose dependent, peak tension was restored to pretreatment control only after the termination of 1 μM, but not 10 or 100 μM Cd^{2+} exposure. The Schild plot suggested that Cd^{2+} and Ca^{2+} are competitive antagonists but two additional observations indicated that noncompetitive mechanisms are also involved: (a) the contour of the tension twitch from the Cd^{2+}-depressed muscle could not be restored to pre-cadmium control by increasing extracellular Ca^{2+} concentration, and (b) the contraction pattern after a reduction in external Ca^{2+} concentration from 2.0 to 0.5 mM differed from that after exposure to 25 μM Cd^{2+}, even though both treatments depressed peak tension development by 80%. When the coronary-perfused rabbit heart was employed, left ventricular Ca concentration was not affected by perfusion with 100 μM Cd^{2+} even though mechanical function was obliterated by this dose. In conclusion, the mechanisms of action of Cd^{2+} are complex and do not appear to involve the displacement of Ca from the cardiac cell.

The depression of myocardial contractile function by cadmium ion (Cd^{2+}) has been observed in heart tissue derived from numerous species (1–7). This inhibitory action has been hypothesized to be related to a competitive interaction of Cd^{2+} with calcium ion (Ca^{2+}) for binding sites involved in the excitation–contraction coupling sequence (3–5, 7). The displacement of Ca^{2+} from the cardiac cell by 0.5–1.0 mM Cd^{2+} (4, 5) appears to provide the most convincing evidence in support of this hypothesis. The present study was designed to determine if the changes in cardiac mechanical function produced by exposure to micromolar concentrations of Cd^{2+} also were related to the loss of cellular Ca^{2+} (4, 5). We also sought to determine if some of the actions of Cd^{2+} could be attributed to noncompetitive components. The isolated, coronary-perfused rabbit heart was employed to study the effects of Cd^{2+} administration on tissue Ca concentration and the isometric kitten papillary muscle was used to study the Cd^{2+}-induced changes in mechanical function.

Methods. *Papillary muscles.* The right ventricular papillary muscle was excised from the heart of the chloroform-anesthetized kitten (340–1450 g) and arranged to contract isometrically in physiologic solution as described previously (8, 9). An internal circulation within the glass superfusion chamber (10) and pH of the bicarbonate-buffered solution of 7.4 were maintained by continuously aerating the bathing fluid with a 95% O_2–5% CO_2 gas mixture (oxygen partial pressure, 620–650 mm Hg). Muscles were stimulated at a constant rate of 20/min by square pulses of 2-msec duration and 1.0- to 2.0-V intensity. Temperature was kept constant

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at 30 ± 0.1°. Tension (T) development (RCA 5734 mechano-electronic transducer) and its electronically derived first derivative (dT/dt) were recorded continuously (Electronics for Medicine, Inc.). During the 3-hr stabilization period following excision, muscle length was adjusted so that active force development was maximal.

Twenty-two papillary muscle preparations were used in three series of experiments. In the first series, the dose-related effects of Cd^{2+} on ventricular muscle function were established by exposing the preparations to 1, 10, or 100 μM $CdCl_2$ for 30 min. Thereafter, the recovery of mechanical function was studied by terminating the Cd^{2+} treatment and restoring Cd^{2+}-free conditions for 90 min.

The antagonistic actions of Ca^{2+} and Cd^{2+} on papillary muscle function were studied in the remaining two series of experiments. In one series, muscles that had been exposed to 10 or 15 μM $CdCl_2$ were subjected to progressive increments in Ca^{2+} concentration until tension development reached pre-cadmium values. The performance parameters (time-to-peak tension, cycle duration) that characterized the tension twitch prior to Cd^{2+} exposure were compared with those after the addition of more Ca^{2+} to the muscle bath and the restoration of tension development. These comparisons were made to determine if the twitch of the Cd^{2+}-depressed muscle could be restored exactly to the control state by increasing external Ca^{2+} concentration.

In the final group of experiments, each muscle was exposed to progressive increases in Cd^{2+} concentration (0, 10, 25, and 50 μM) and, at each dose, stepwise changes in Ca^{2+} concentration were produced. We analyzed these data to determine if, for comparable decreases in peak tension, the twitch in low Ca^{2+} solution was superimposable with the twitch in the presence of Cd^{2+} and with Ca^{2+} at its control level. These data also were used to help determine the type of antagonism that existed between Ca^{2+} and Cd^{2+}. Log $[Ca^{2+}]$ versus peak tension curves were constructed and dose ratios (11) determined. The Ca^{2+} dose ratio, X, is here defined as the quotient: $[Ca^{2+}]_{wo}/[Ca^{2+}]_w$, where $[Ca^{2+}]_{wo}$ is the extracellular Ca^{2+} concentration associated with a chosen level of tension development without Cd^{2+} present, and $[Ca^{2+}]_w$ is the extracellular Ca^{2+}concentration causing the same level of tension development with Cd^{2+} present. The dose ratios were used to plot the relationship between log $(X - 1)$ and $-\log [Cd^{2+}]$. This procedure of Schild (12, 13) is used to determine if two substances such as Ca^{2+} and Cd^{2+} are competitive or noncompetitive antagonists.

Each muscle was assumed to be cylindrical and to possess a specific gravity of unity so that muscle diameter ($2r$) and cross-sectional area (πr^2) could be calculated from muscle length (L) and weight (W) measurements with the following equation: $\pi r^2 = W/L$. Tension development was normalized by muscle cross-sectional area. The mean diameter (±SE) of all papillary muscles employed in this study was 0.71 ± 0.04 mm.

Rabbit hearts. The white, New Zealand rabbit (2–3 kg) was thoracotomized following anesthetization with intravenous sodium pentobarbital (40 mg/kg) and onset of positive pressure ventilation with 100% O_2. The aorta was cannulated retrogradely for coronary artery perfusion after administration of heparin (1000 units) and the heart excised as described previously (8). The atrioventricular node was crushed and the ventricle paced at 42 beats/min by square pulses of 4-msec duration and 4-V intensity (Grass, S44). A fluid-filled balloon connected to a pressure transducer (Gould-Statham, P23db) was passed into the left ventricle through a small incision made in the left atrium and balloon volume was adjusted so that diastolic pressure was about 10 mm Hg. Aortic perfusion pressure and left ventricular isovolumic pressure were recorded continuously (Gould polygraph, 2600). Perfusate flow rate of 25 ml/min and temperature of 25° were maintained constant throughout each experiment.

Left ventricular Ca concentration was measured at 5 and 30 min after onset of perfusion with 100 μM Cd^{2+} and also in un-

TABLE I. PERFORMANCE OF PAPILLARY MUSCLES IMMEDIATELY PRIOR TO Cd^{2+} TREATMENT

Condition	Developed tension (g/mm^2)	$+dT/dt_{max}$ ($g/mm^2/sec$)	$-dT/dt_{max}$ ($g/mm^2/sec$)	TTP (msec)
Before 1 μM Cd^{2+}	4.6 ± 1.1	18.1 ± 4.5	20.7 ± 5.2	467 ± 3
Before 10 μM Cd^{2+}	4.6 ± 0.8	19.3 ± 3.3	19.4 ± 3.2	436 ± 9
Before 100 μM Cd^{2+}	3.3 ± 0.2	13.6 ± 1.2	15.6 ± 2.6	421 ± 15

treated control hearts. Perfusion duration was 90 min in both treated and untreated hearts. Calcium was extracted from each of several left ventricular samples (300–400 mg) with equal parts of glacial acetic acid and trichloroacetic acid as described by Sparrow and Johnstone (14) and the Ca concentration in the tissue extract was quantitated by atomic absorption spectrophotometry (Varian, AA-375). Thirty-eight hearts were studied with these methods.

Radioisotopic calcium (^{45}Ca, New England Nuclear) was used to provide an alternate method for assessing the effect of 100 μM Cd^{2+} treatment on tissue Ca concentration in an additional 12 experiments. The isolated hearts were perfused for 90 min with solution containing ^{45}Ca and then, without a change in perfusate ^{45}Ca specific activity, 100 μM Cd^{2+} perfusion was initiated and maintained for 30 min. After treatment of perfusate samples (0.2 ml) and left ventricular tissue samples (100–200 mg) for 12 hr in 1.5 ml of Soluene-350 (Packard Instrument Co.), 15 ml of toluene with PPO (6.20 g/liter) and POPOP (0.14 g/liter) was added to each sample. Liquid scintillation spectrometry (Beckman LS 9000) was employed to quantitate the activity of ^{45}Ca in perfusate and tissue. Tissue Ca concentration was calculated assuming that the specific activity of ^{45}Ca in the tissue was equal to the specific activity of ^{45}Ca in the perfusate.

Statistical analysis. Student's *t* test was employed to determine the statistical significance of the differences between individual pairs of mean values. Values in figures, tables, and text are reported as means ± SE.

Results. *Papillary muscles.* Control values for developed tension, maximum rate of contraction ($+dT/dt_{max}$), maximum rate of relaxation ($-dT/dt_{max}$), and time-to-peak tension (TTP) are shown in Table I for those muscles that were treated with 1, 10, or 100 μM $CdCl_2$. Subsequent exposure of the papillary muscle to Cd^{2+} produced a dose-dependent depression of mechanical performance that was associated with proportional decreases in developed tension, $+dT/dt_{max}$ and $-dT/dt_{max}$ (Fig. 1). However, TTP was unaffected by exposure of the papillary muscle to either 1 or 10 μM Cd^{2+} solution, but decreased significantly when Cd^{2+} concentration was 100 μM (Fig. 1). This decrease in TTP during exposure to 100 μM Cd^{2+} was associated with a shortened duration of the contraction–relaxation cycle as illustrated in the right panel of Fig. 2.

The extent of recovery of mechanical function following the restoration of Cd^{2+}-free conditions after 30 min of Cd^{2+} expo-

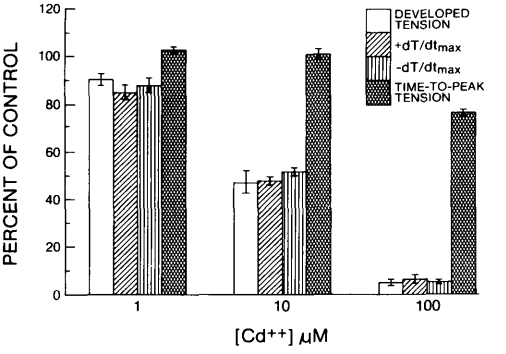

FIG. 1. Depression of papillary muscle function by Cd^{2+}. The dose-dependent decreases in developed tension were paralleled by decreases in both $+dT/dt_{max}$ and $-dT/dt_{max}$. Time-to-peak tension was unaffected by the 1 or 10 μM Cd^{2+} dose but was significantly depressed ($P < 0.005$) after onset of 100 μM Cd^{2+} treatment.

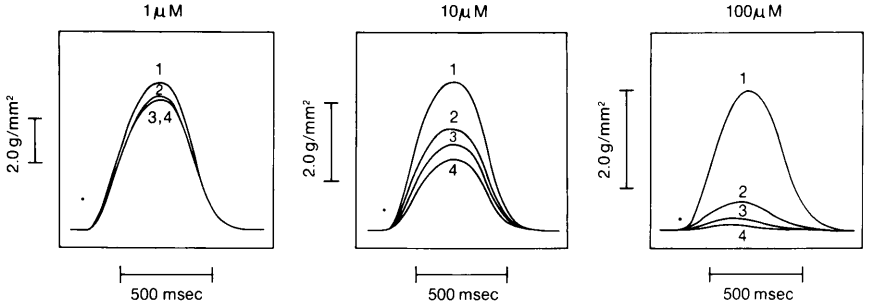

FIG. 2. Effect of Cd^{2+} on tension development by the kitten papillary muscle. Shown in each panel are tension twitches immediately before (trace 1) and 5, 15, and 30 min after (traces 2, 3, and 4) Cd^{2+} treatment. Both the mechanical cycle duration and the TTP were attenuated by the 100 μM Cd^{2+} dose (right panel), but not by the 1 μM (left panel) or 10 μM (middle panel) doses. Papillary muscle diameters were 0.53 mm (left panel), 0.51 mm (middle panel), and 0.67 mm (right panel). The stimulus artifact is shown by the blip in the lower left quadrant of each panel. These tracings were redrawn from photographs of oscillographic records.

sure was dependent on the prior Cd^{2+} treatment dose (Fig. 3). Force development by muscles previously exposed to 1 or 10 μM Cd^{2+} returned rapidly toward control levels after the onset of Cd^{2+}-free superfusion, but recovery was slow and reached only 26% of control after restoration of Cd^{2+}-free conditions following 100 μM Cd^{2+} treatment.

When the effects of Ca^{2+} and Cd^{2+} on papillary muscle function were compared, it was apparent that neither substance antagonized exactly the actions of the other. Increasing Ca^{2+} in the bathing solution of the Cd^{2+}-depressed muscle led to a restoration of tension development to the pre-cadmium control level, but the pre- and post-treatment twitches were not superimposable as illustrated by the different TTPs (Table II). Furthermore, the changes in papillary muscle function following a reduction in extracellular Ca^{2+} concentration did not always mimic those after Cd^{2+} administration. There was a decrement in

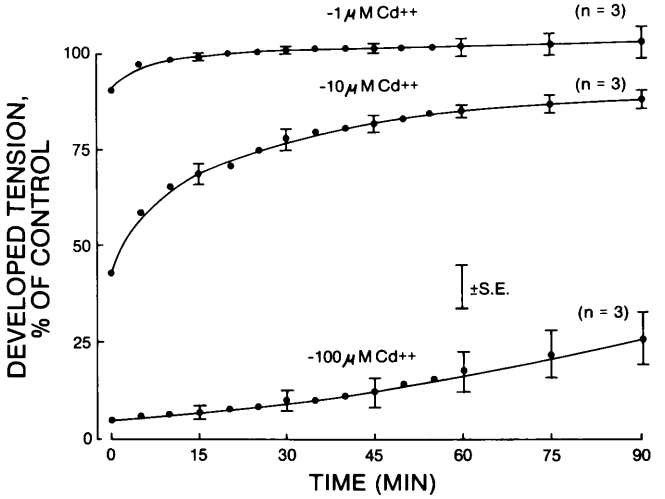

FIG. 3. Recovery of papillary muscle function following restoration of Cd^{2+}-free superfusion after 30 min of Cd^{2+} treatment. Developed tension reached 103 ± 4% of control during recovery after 1 μM Cd^{2+} treatment (upper curve) and 86 ± 3% of control after 10 μM Cd^{2+} (middle curve), but was only 26 ± 7% (lower curve) of control during recovery after treatment with 100 μM Cd^{2+}.

TABLE II. EFFECT OF INCREASED EXTRACELLULAR Ca^{2+} ON TIME-TO-PEAK TENSION DEVELOPMENT (TTP) BY THE Cd^{2+}-DEPRESSED PAPILLARY MUSCLE

$[Ca^{2+}]_o$ (m*M*)	$[Cd^{2+}]_o$ (μM)	Tension development (g/mm²)	TTP (msec)
2.0	0	4.06 ± 0.49	430 ± 16
2.0	10–15	1.17 ± 0.23*	432 ± 19
5.0–6.0	10–15	4.02 ± 0.70	365 ± 27**

* $P < 0.001$.
** $P < 0.05$.

TTP when the muscle was exposed to 25 μM Cd^{2+} but not when Ca^{2+} concentration was reduced to 0.5 m*M*, even though the decreases in tension development produced by these two treatments were identical (Fig. 4). The duration of the mechanical cycle also was decreased by exposure to 25 μM Cd^{2+} but not by decreasing Ca^{2+} to 0.5 m*M*. The contraction pattern dissimilarities that resulted from 25 μM Cd^{2+} versus 0.5 m*M* Ca^{2+} treatments were not observed when the effects of smaller concentrations of Cd^{2+} were compared with the effects of smaller decrements in Ca^{2+}, provided the changes in peak tension were similar. As shown in Fig. 4, neither 1.0 m*M* Ca^{2+} exposure nor 10 μM Cd^{2+} treatment produced a shift in TTP. Contraction cycle duration also remained constant.

Schild's plot (12, 13) demonstrated a linear relationship between log $(X - 1)$ and $-\log [Cd^{2+}]$ (Fig. 5). The $-\log [Ca^{2+}]$ versus peak tension plots, from which the Ca^{2+} concentrations for the dose ratio calculations were derived, were approximately parallel over the range of Cd^{2+} concentrations tested. The Ca^{2+} concentrations that produced an interpolated peak tension of 3 g/mm² were selected for the dose ratio calculations. This value was approximately one-half of the maximum peak tension observed for each dose–response relationship.

Rabbit hearts. Left ventricular Ca con-

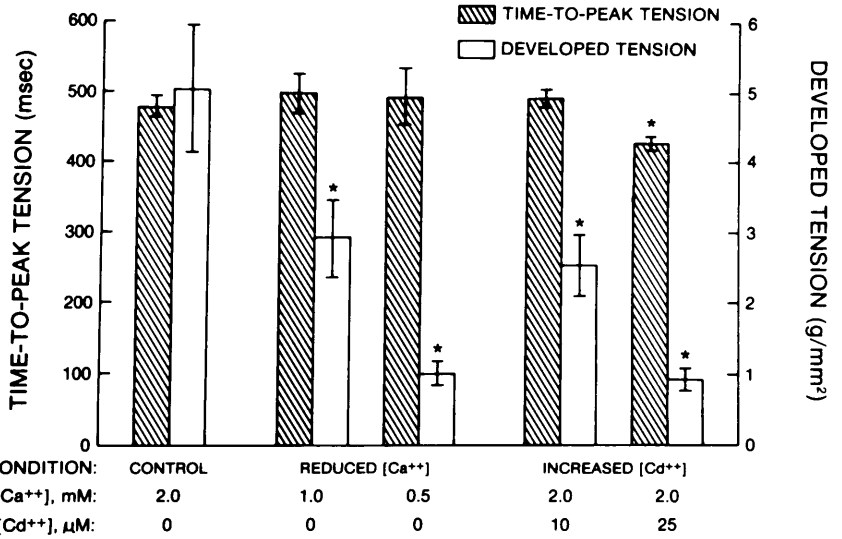

FIG. 4. Comparison of the papillary muscle response after reduced Ca^{2+} with that after increased Cd^{2+}. Administration of 25 μM Cd^{2+} produced an 80% reduction in developed tension and a 10% decrease in TTP, whereas the decrement in external Ca^{2+} from 2.0 to 0.5 m*M* also depressed tension development by 80%, but produced no change in TTP. In contrast, developed tension was depressed to the same extent by 10 μM Cd^{2+} treatment as it was by 1.0 m*M* Ca^{2+} exposure, but TTP was not affected by either of these interventions. Starred (*) values are significantly different from control.

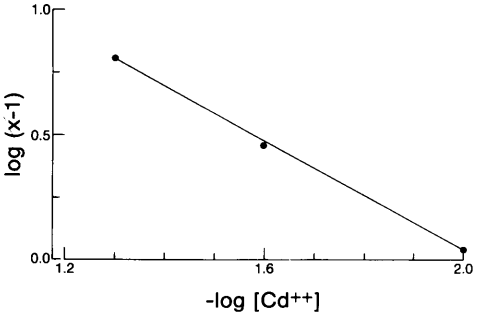

FIG. 5. Equieffective dose test for competitive antagonism. Log dose–response curves from five experiments were used to obtain the Ca^{2+} concentrations for the dose ratio, X, calculations.

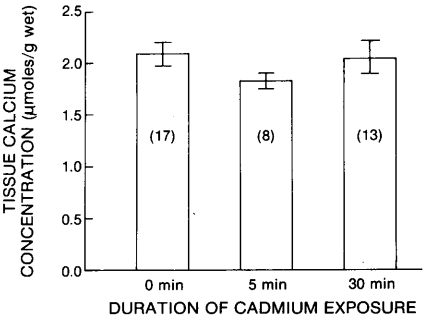

FIG. 6. Effect of 100 μM Cd^{2+} perfusion on rabbit heart Ca concentration. Measurement by atomic absorption spectrophotometry of Ca concentrations in extracts prepared from left ventricular tissue samples showed that there was no change in left ventricular Ca concentration after onset of Cd^{2+} perfusion. Numbers of experiments are shown in parentheses.

centration was unaffected by 100 μM Cd^{2+} perfusion (Fig. 6) although this dose produced a decrease in left ventricular pulse pressure (systolic pressure minus diastolic pressure) from 130 ± 1 to 6 ± 1 mm Hg at 5 min and to zero mm Hg at 30 min. Furthermore, the values for tissue Ca concentration obtained by atomic absorption spectrophotometry (Fig. 6) were essentially the same as those obtained with liquid scintillation spectrometry of ^{45}Ca (Table III).

Discussion. The depression by Cd^{2+} of myocardial contractility shown in this and previous studies (1–7) has been hypothesized to occur as a consequence of Cd^{2+} competition with Ca^{2+} at critical sites in the excitation–contraction coupling mechanism (2, 3, 7). The linear relationship between log (X − 1) and −log [Cd^{2+}] (Fig. 5) also suggests, but does not prove (13), that Cd^{2+} competitively inhibits the actions of Ca^{2+}. Nevertheless, despite the apparent competitive antagonism of Ca^{2+} by Cd^{2+} suggested by the results of this and other studies (2, 3, 7), there was no measurable loss of Ca^{2+} from the left ventricle of the isolated rabbit heart following the onset of 100 μM Cd^{2+} administration. This finding was surprising in view of the previously observed Cd^{2+}-induced displacement of Ca^{2+} from the cultured rat heart cell after onset of 0.5 to 1.0 mM Cd^{2+} superfusion (4, 5). Apparently, treatment of cardiac tissue with these larger Cd^{2+} concentrations stimulates Ca^{2+} displacement from cardiac tissue. However, although higher levels of external Cd^{2+} probably dislodge Ca^{2+} from the sarcolemma and other sites within the cardiac cell, the smaller 0.1 mM dose that we employed was not effective in displacing detectable quantities of Ca^{2+} from the tissue even though mechanical function was obliterated. We conclude that the Cd^{2+}-induced depression of myocardial contractility was unrelated to the loss of Ca^{2+} from the myocardial cell.

Furthermore, our data suggest that the action of Cd^{2+} on ventricular function includes a component that is independent of competitive mechanisms. There was a failure to exactly reverse the contraction pat-

TABLE III. LEFT VENTRICULAR Ca CONCENTRATIONS DETERMINED FROM TISSUE ^{45}Ca ACTIVITY MEASUREMENTS

Perfusate Cd^{2+} concentration (μM)	Tissue Ca concentration (μmole/g wet wt)	Left ventricular pulse pressure[a] (mm Hg)
0 (n = 6)	1.96 ± 0.10	111 ± 5
100 (n = 6)	2.29 ± 0.15	Negligible

[a] Systolic pressure minus diastolic pressure.

tern of the Cd^{2+}-depressed papillary muscle by increasing extracellular Ca^{2+}, and the contour of the papillary twitch following 25 μM Cd^{2+} differed from the contour of the twitch after Ca^{2+} was reduced to 0.5 mM, even though each of these interventions depressed peak tension to the same extent. Neither response would have been predicted if only competitive mechanisms were operative. These data, however, do not negate the possibility that Ca^{2+} and Cd^{2+} compete for common receptors. Indeed, the competition between Cd^{2+} and Ca^{2+} for contraction-related binding sites may play a prominent role in the Cd^{2+}-induced depression of cardiac mechanical function. We wish to suggest that noncompetitive mechanisms also are involved.

The shortened TTP and cycle duration that occurred in the papillary muscle twitch after 25–100 μM, but not after 1 or 10 μM Cd^{2+} treatment (Figs. 1, 2, and 4) suggest that additional subcellular systems were affected when the tissue was exposed to the higher Cd^{2+} concentrations. This possibility is supported by our additional observation that tension development by the papillary muscle was reversed with relative ease following restoration of Cd^{2+}-free conditions after 1 and 10 μM Cd treatment but was only partially and slowly reversed following restoration of Cd^{2+}-free superfusion after 100 μM Cd^{2+} exposure (Fig. 3). Toda (7) showed that myocardial function during exposure to 20 μM Cd^{2+} was restored to control levels by raising extracellular Ca^{2+} concentration, whereas the restoration of tension development to control during 100 μM Cd^{2+} treatment could be achieved only by adding cysteine to the external solution. It was suggested (7) that cysteine reverses the Cd^{2+} antagonism of contractile protein function produced by higher Cd^{2+} doses.

In summary, it is probable that the depressant action of Cd^{2+} on cardiac ventricular function includes both competitive and noncompetitive components, but the relative importance of each cannot be assessed from our data. However, the competitive actions of Cd^{2+} were not sufficient to displace Ca^{2+} from the ventricular cell. The largest Cd^{2+} dose we employed did not dislodge detectable quantities of Ca^{2+} from the isolated rabbit heart even though there was a cessation of mechanical activity. Furthermore, when cardiac tissue was exposed to the higher concentrations of Cd^{2+}, additional cellular systems appeared to be significantly affected by Cd^{2+} as illustrated by the shortening of both TTP and the duration of the mechanical cycle.

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