Effects of Cadmium on the Contractility of a Frog Cardiac Muscle in Relation to pH of External Solution

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Abstract In order to examine the pH dependency of Cd effects on cardiac muscle, the electrical and mechanical activities in the bullfrog heart were investigated in relation to various external pHs and concentrations of Cd. The amplitude and duration of the action potential or the spontaneously beating frequency in the atrium were not significantly affected during the 3-min administration of various concentrations of Cd in a range from pH 6 to 10, but the isotonic contraction at the end of the same period was greatly altered: the lower the external pH the more Cd decreased the contractility of the atrium. This pH dependency of Cd effects was also observed in SO_4 -Ringer's solution whose anions, SO_4 , were more impermeable than Cl. Decrease in contractility in Cd-Ringer's solution was counteracted by excess Ca. The lower the pH of the Cd-Ringer's solution, the more Ca was necessary to counteract an equal amount of Cd. The amount of Cd-uptake into the atrium was analyzed after soaking the atrium in various concentrations of Cd-Ringer's solution. The higher the concentration of external Cd, the larger the Cd-uptake into the atrium. No pH dependency, however, was observed in this relationship. This suggests that probably only a small fraction of total Cd-uptake interacts with Ca-binding sites which is specific to contraction, and causes the Cd-induced decrease in contractility. This process is considered to be pH-dependent. However, most Cd-uptake into cardiac muscle is pH-independent and may be non-specific to contraction.

Effects of cadmium (Cd) on the electrical and mechanical activities of cardiac muscle in normal pH solution have been studied (KLEINFELD *et al.*, 1955; TAKAHASHI *et al.*, 1956; KLEINFELD and STEIN, 1968; HAYASHI and HORIUCHI, 1971a; TODA, 1973). It was found that a low concentration of Cd (about $10\text{ }\mu\text{M}$) brought about a decrease in contractility without an appreciable change in the action potential, and this Cd effect on contractility was completely antagonized by excess Ca (HAYASHI and HORIUCHI, 1971a). It was also reported

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that, when the pH of the medium was changed, the amount of bound Ca in isolated sarcolemma or sarcoplasmic reticulum, which is closely related to contraction, was changed (NAKAMARU and SCHWARTZ, 1970; WILLIAMSON *et al.*, 1975). Based on these facts, it was postulated that Cd which counteracted Ca might also exert pH dependency; *i.e.*, the lower the external pH of the Ringer's solution, the more enhanced the Cd effects. The everted sinus-atrium preparation of a bullfrog was used to test this assumption. Recording of intracellular potential of the excised atrium was made to confirm the results obtained from the sinus-atrium preparation.

Finally, the relation between the amount of Cd-uptake and the contractility was discussed in relation to the external pH.

A preliminary account of this work has been published elsewhere (HORIUCHI and HAYASHI, 1978).

MATERIALS AND METHODS

The everted sinus-atrium preparations of bullfrogs (*Rana catesbeiana*) of either sex were used. The frogs were anesthetized by an intrathecal injection of 0.5 ml urethane solution (0.25 g/ml). The heart was excised together with its blood vessels which were ligated and sectioned proximal to the heart. The greater part of the ventricle was removed by cutting out about 2 mm below the atrio-ventricular groove. The remaining heart (atrium with sinus) kept beating in an almost normal rhythm. After washing out the blood in the heart, most of the septum in the atrium was removed with great care. The sinus-atrium preparation was reversed inside out by a puff of Ringer's solution from a pipette. This everted sinus-atrium preparation was fixed tightly at the tip (3.3 mm in inner diameter) of a cannula (30.0 mm in inner diameter) with a piece of thread, and was immersed in Ringer's solution of various pHs (30 ml). Normal Ringer's solution of pH 7.2 was put into the cannula, and the inside pressure was 1 cmH₂O higher than that outside. The epicardium of the atrium was much thicker than the endocardium (HORIUCHI and HAYASHI, 1973); therefore the everted sinus-atrium preparation may allow a quick access and wash-out of drugs by exchanging the outside solution (HAYASHI and HORIUCHI, 1971b). Figure 1 illustrates the schematic diagram for simultaneous recording of mechanical and electrical events of the preparation. The contraction of the preparation was transformed into a small change in pressure, and recorded with a high sensitivity pressure transducer (Nihon Kohden LPU-0.1), a carrier amplifier (AP-300 G), and a pen writer (PMP-3004). The electrical events were recorded by a suction electrode (200 μ m inner tip diameter) and an amplifier of a high input impedance (AB-300 G; ARITA and HAYASHI, 1973). In order to confirm the findings by using suction electrodes, we recorded intracellular membrane potentials by a conventional method (microelectrodes of more than 20 M Ω

Fig. 1. Apparatus for simultaneous recording of electrical and mechanical activities of an everted sinus-atrium preparation.

Table 1. Composition of Ringer's solution (in mm).

	Normal R*	Control R (Cl-R)	SO ₄ -R
NaCl	110	90	0
Na ₂ SO ₄	0	0	45
KCl	2	2	0
K ₂ SO ₄	0	0	1
CaCl ₂	1	1	0
Ca gluconate	0	0	1
Glucose	10	10	10
Sucrose	0	0	61
Tris/HCl**	10	30	0
Tris/H ₂ SO ₄	0	0	30
pH	7.2	6.5-10.0	6.5-9.0

* R: Ringer's solution.

**Buffer solution for pH 10 Ringer's solution was glycine-NaOH instead of Tris-HCl.

filled with 3 M KCl; a preamplifier, Nihon Kohden MZ-4; a cathode-ray oscilloscope, AVB-8; and a kymograph camera, PC-2B).

The composition of Ringer's solution is shown in Table 1. The Ringer's solution of various pHs (pH 6-10) was obtained by adjusting the amount of HCl (Toa Electronics pH meter HM-6A). Cd-containing Ringer's solution was made by addition of a small amount of concentrated Cd solution to the

Cd-free Ringer's solution of various pHs. No change in pH of the solution was observed by addition of Cd.

All the experiments were carried out at room temperature (20–24°C).

Quantitative analysis of Cd in atrial muscle was carried out on the following preparations. Epicardium of an excised atrium was removed with a fine forceps in order to eliminate a thick non-muscular component of the epicardium (HORIUCHI and HAYASHI, 1973). Cadmium of various concentrations was applied to the spontaneously beating atrium in Ringer's solution of either pH 6.5 or pH 9.0 for 3 min. The atrium was blotted immediately with a filter paper, weighed, then ashed with a plasma reactor (Yamato PR-302) at about 100°C for 12 hr (O_2 supply, 40 ml/min; power, 120 W). The ashed atrium was dissolved in 0.15 ml of 0.5 N HCl, and the resulting solution was diluted with distilled water to make up to 10 ml. Its Cd concentration was determined by atomic absorption spectrophotometry (Hitachi 508 A) using a standard additional method.

RESULTS

Effects of various pHs and Cd concentrations on contraction

In the previous experiment on a bullfrog sinus-atrium preparation, it was shown that Cd of $13 \mu\text{M}$ in normal Ringer's solution (pH 7.2) decreased the contractility of the cardiac muscle to almost half without an appreciable change in its membrane potential (HAYASHI and HORIUCHI, 1971a). In the present experiment it was found that if the pH of the Ringer's solution was raised, this Cd effect was reduced. A typical example is shown in Fig. 2. First, in the

Fig. 2. Effects of various pHs on the contractility of atrial muscle in the presence or absence of Cd ($13 \mu\text{M}$). Contraction is expressed as a percentage of that in pH 7.2 control Ringer's solution.

pH 7.2 control Ringer's solution we started to measure cardiac output, which was the indicator of isotonic contraction, then we applied $13\text{ }\mu\text{M}$ Cd to the preparation by adding a small amount of concentrated CdCl_2 into the perfusing solution. Recording was made 3 min after the application of Cd, and the preparation was washed with 300–600 ml (10–20 times the chamber volume) of the pH 7.2 control Ringer's solution. After such washing, the contractility was almost restored to the initial control value. The control Ringer's solution of a different pH was then tested, and so forth. All the data were standardized by contraction in the pH 7.2 control Ringer's solution.

When the pH of the control Ringer's solution increased from 7.2 to 10.0, the contractility increased only slightly, *i.e.*, to 104.6% in this case (contractility in the latter pH solution divided by that in the former).

On the other hand, the effects of Cd were dramatically altered as the pH of the external solution changed, *i.e.*, Cd decreased the contractility to 34.4% of control in pH 7.2 solution, but to only 90.6% in pH 10.0 solution, in this case. The extent of the $13\text{ }\mu\text{M}$ Cd-induced decrease in contractility varied from preparation to preparation, but Cd was always more effective in a lower pH solution than in a higher one (35–55% of control in pH 7.2 solution, and only 85–95% in pH 10.0 solution).

The effects of various concentrations of Cd on the contractility of atrial muscle in sinus-atrium preparations in pH 9.0 and pH 6.5 Ringer's solution are shown in Fig. 3. Each measurement was made after the preparation had been washed out by a large amount of Ringer's solution, and all data were expressed as percentages of those before each application of Cd. Both in the higher concentration of Cd and in the lower pH solution, the contractility

Fig. 3. Effects of various concentrations of Cd for 3 min on the contractility of atrial muscle in relation to pH (6.5 and 9.0). The contraction is expressed as a percentage of that before each application of Cd. Vertical bars represent standard error of means (5 cases).

of atrial muscle was decreased; the contractility decreased to almost zero in 100 μM Cd-pH 6.5 Ringer's solution, but about 40% of control contraction remained in 100 μM Cd-pH 9.0 Ringer's solution. From another viewpoint, 100 μM Cd in pH 9.0 brought about almost the same effect on contraction as one-tenth the Cd (10 μM) in a pH 6.5 solution.

Effects of Cd on the electrical activity in various pH solutions

Figure 4 illustrates the effects of 13 μM Cd on the duration of action potential and the interval of spontaneous beat of everted sinus-atrium preparations in various pH Ringer's solutions. The action potential was obtained by using a suction electrode. The duration of the action potential was defined as the duration at the level of 10% of the amplitude of the action potential. All the data were standardized with those in pH 7.2 control Ringer's solution. The extent of change in the action potential duration and the cardiac interval with change in the pH of the external solution varied between preparations. For the present purpose of investigating the pH dependency of the Cd effect on contraction, a preparation was selected whose duration and cardiac interval was hardly altered by pH change since changes in these parameters *per se* would affect the contractility. Cd increased both the interval and duration of the action potential very slightly in the whole range of pH (the difference was less than 10% of the control), but no pH-dependent Cd effect was observed which was quite different from Cd effects on contraction.

Fig. 4. Effects of various pHs on cardiac intervals and durations of action potential recorded by using a suction electrode with or without Cd (13 μM) for 3 min. The cardiac interval and duration are expressed as percentages of those in pH 7.2 control Ringer's solution.

Fig. 5. Effects of Cd on the intracellular membrane potential of a spontaneously beating atrial muscle. A: membrane potential in control Ringer's solution of pH 6.5 (Cd-free). B: that of atrium treated with Cd of $30 \mu\text{M}$ for 3 min (pH 6.5). Note no appreciable changes in resting or action potential were observed by application of Cd.

The above-stated results obtained by a suction electrode were tested by using an intracellular microelectrode technique. The sinus-atrium preparation was fixed, with the endocardial face upward, on a cork plate. A typical example of a record obtained from an excised atrium with sinus is shown in Fig. 5. First, the action potential of a spontaneously beating atrium was recorded intracellularly in pH 6.5 control Ringer's solution (Fig. 5A). Then, this control solution was substituted by $30 \mu\text{M}$ Cd-pH 6.5 Ringer's solution. After the application of Cd for 3 min (namely, when the mechanical recording was made), the membrane potential was again recorded (Fig. 5B). Cd in pH 6.5 Ringer's solution caused a very slight change in the spike amplitude, duration of action potential, resting potential and cardiac interval (102, 100, 104 and 101% respectively, in this case), while contractility under the binocular microscope had almost disappeared.

The results in pH 9.0 Ringer's solution were similar to those in pH 6.5 solution; *i.e.*, Cd of $30 \mu\text{M}$ for 3 min caused no appreciable change in the spike amplitude, duration of action potential, resting potential, or cardiac interval (the difference was less than 2% of the control) while contractility decreased slightly.

The parameters stated above were not greatly affected (less than 5%) by simple alteration of the external pH of the control Ringer's solution from 6.5 to 9.0.

Influence of anion on pH-dependent Cd effects

Chloride ion is known as a carrier of ionic current during a cardiac action potential. Hence, the possibility was examined of whether the pH-dependent Cd effects on contraction were coupled with anion. This was tested by substituting Cl for SO_4 which is thought to be an anion that does not permeate the cel-

Fig. 6. Effects of Cd ($13 \mu\text{M}$, 3 min) on the contractility of atrial muscle (A), cardiac interval (B) and duration of action potential (C) in relation to pH and anion species (Cl and SO_4). Data are expressed as percentages of those before each application of Cd.

lular membrane. The action potential was recorded by a suction electrode. Figure 6 illustrates the Cd effects ($13 \mu\text{M}$, 3 min) on contraction (A), cardiac interval (B) and duration of action potential (C) in Cl- or SO_4 -Ringer's solution in relation to pH (6.5 and 9.0). All the data were expressed as percentages of those prior to each application of Cd. Although Cd did not cause changes in the cardiac interval or duration of action potential despite the alteration of anion species and pH, a remarkable pH dependency of the Cd effect on contraction was observed in both Cl- and SO_4 -Ringer's solution; *i.e.*, the lower the external pH, the more pronounced the effects of Cd on contraction. The rate of Cd-induced decrease in contractility in SO_4 -Ringer's solution was much greater than that in Cl-Ringer, irrespective of pH.

pH-dependent antagonistic action of Ca against Cd

Figure 7 shows an example of antagonism between Cd and Ca on contraction of atrial muscle of a spontaneously beating everted sinus-atrium preparation. Cd of $10 \mu\text{M}$ in pH 6.5 Ringer's solution brought about a marked decrease in contractility (A_1). When the contraction attained 50.7% of the control, 3 min after application of Cd, a further 1.5 mM Ca was added to the solution (final Ca concentration was 2.5 mM). The contraction was restored to 88.6% of the control 2.5 min later (A_2). Cd of $10 \mu\text{M}$ in pH 9.0 Ringer's solution for 3 min slightly decreased the contraction of atrial muscle (85.3% of the control) (B_1). A further addition of 0.3 mM Ca (final Ca concentration was 1.3 mM) almost restored the contraction (91.1% of the control) (B_2). The amount of Ca, which was added to the solution for complete restoration of twitch

Fig. 7. Antagonistic action of Ca against Cd on the contractility of atrial muscle. A_1 and A_2 : experiments in pH 6.5 Ringer's solution. B_1 and B_2 : those in pH 9.0 solution. Applications of Cd ($10 \mu\text{M}$) are indicated by downward arrows in A_1 and B_1 . Additions of Ca to Cd-Ringer (3 min after application of Cd) are indicated by downward arrows in A_2 and B_2 .

Fig. 8. Antagonistic action of Ca against Cd on the contractility of atrial muscle. A_1 and A_2 : experiments in pH 6.5 Ringer's solution. B_1 and B_2 : those in pH 9.0 solution. Cd of $30 \mu\text{M}$ in A_1 and B_1 , and $30 \mu\text{M}$ Cd with high Ca in A_2 and B_2 were applied throughout the period indicated by horizontal bars.

contraction, was larger in low pH Cd-Ringer's solution than in high pH Cd-solution (Cd concentration was the same). The ratio of the added amount of Ca sufficient to counteract Cd in pH 6.5 solution to that in pH 9.0 solution was approximately 5 ($=1.5 \text{ mM}/0.3 \text{ mM}$). The ratio was 2 for final Ca concentrations). A similar antagonism between Cd and Ca was also observed for a high concentration of Cd (30, 100 and $300 \mu\text{M}$).

Antagonism between Cd and Ca on the contractility of cardiac muscle was examined in a different method. Figure 8 shows the effects of either Cd-Ringer's solution or (Cd+high Ca)-Ringer's solution on the contractility of atrial muscle of the everted sinus-atrium preparation. Cd of $30 \mu\text{M}$ in pH 6.5 Ringer's solution for 2.5 min markedly decreased the contractility (45.7% of the control) (A_1). Despite containing the same concentration of Cd, however, simultaneous application of Cd and high Ca (5 mM Ca instead of 1 mM Ca in control solution) in pH 6.5 Ringer's solution made no appreciable change in contractility (96.8% of the control 3 min after application of Cd+high Ca mixture) (A_2). The same type of experiments were repeated in pH 9.0 Ringer's solution (B_1 and B_2). The amount of Ca which prevented the Cd-induced decrease in contractility was much less in pH 9.0 solution than in pH 6.5 solution (the ratio of the latter to the former was approximately 3.3 in this case).

Uptake of Cd into heart

Various concentrations of Cd were administered to the atria in either pH 6.5 or pH 9.0 Ringer's solution for 3 min. Then, the amount of Cd-uptake into cardiac tissue was measured by atomic absorption spectrophotometry. Figure 9 represents the relationship between the amount of Cd in the heart and the external Cd concentration in pH 6.5 (closed circle) and pH 9.0 (open circle)

Fig. 9. Amount of Cd-uptake into atrium as a function of external Cd concentration in pH 6.5 (closed circle) and pH 9.0 (open circle) Ringer's solution (mean $\pm$ SEM, 3 cases for each set of data except 6 cases for $100 \mu\text{M}$ Cd-Ringer's solution).

Ringer's solution. Let Cd-uptake into heart and Cd concentration in Ringer's solution be y and x , respectively. Figure 9 shows that the higher " y " is, the higher " x " will be. The parameters of the experimental equation ($y=a \cdot x^b$) are as follows: " a " and " b " are 7.34 and 0.75 in pH 6.5 solution, and 13.1 and 0.63 in pH 9.0 solution, respectively. The null hypothesis that these two experimental curves are equal cannot be discarded by a statistical method, because the slope of two linear regression lines in Fig. 9 are almost equal, and the vertical distance between these lines at the mean value of $\log x$ is almost zero. This signifies that no pH dependency was observed in the relationship between Cd-uptake in the heart and the external Cd concentration in Ringer's solution, even though contractility in low pH solution was markedly decreased by external Cd (*cf.*, Fig. 3). Cd in external Ringer's solution invades not only the interstitial fluid but also the cardiac tissue, because the concentration of Cd taken up into the heart is higher than that in the external solution (see DISCUSSION).

DISCUSSION

In this paper, we have reported for the first time that the negative inotropic effect of Cd on cardiac muscle in pH 7.2 Ringer's solution was dramatically enhanced in acid Ringer's solution without appreciable change in action potential. A few investigators (KLEINFELD *et al.*, 1955; TAKAHASHI *et al.*, 1956; KLEINFELD and STEIN, 1968; HAYASHI and HORIUCHI, 1971a; TODA, 1973) have hitherto described the effects of Cd on the cardiac muscle, but none has tried to study the Cd effect in relation to pH.

The negative inotropic effect on cardiac muscle in acid Ringer's solution *per se* has been studied by many investigators since GASKELL (1880) noticed this phenomenon. There are at least two possible mechanisms underlying the effect of acidosis: (1) acid Ringer causes a change in the action potential which might be due to a decrease in Ca influx, and (2) hydronium ion acts on sarcolemmal or intracellular Ca binding sites, thus decreasing the concentration of free Ca around the myofilaments upon contraction. Acid Ringer's solution caused the following change in the action potential: decrease in rate of rise of the action potential, decrease in slow Na and Ca current, and a variable change in the action potential duration (HECHT and HUTTER, 1965; LORKOVIĆ, 1966; VAUGHAN WILLIAMS and WHYTE, 1967; VAN BOGAERT and CARMELIET, 1972; WADA and GOTO, 1975; POOLE-WILSON and LANGER, 1975; CHESNAIS *et al.*, 1975), and an increase in heart rate due to change in i_{K_2} in alkaline solution (VAN BOGAERT *et al.*, 1978). The reason that we did not observe the appreciable change in action potential with a change in the external pH is this: (1) We changed the pH of the external solution by Tris/HCl buffer which caused a minor change in internal pH, and the change in external pH alone was considered to be less effective on cardiac muscle (LORKOVIĆ, 1966; CINGOLANI *et al.*, 1970).

In most experiments which caused the change in action potential in acid solution, pH was altered by changing the external CO_2 concentration which in turn brought about a decrease in internal pH. (2) Acidity-induced decrease in Ca current may be so small that seemingly no appreciable change was observed in the total shape of the action potential. KOHLHARDT *et al.* (1976) observed a decrease in Ca current with decrease in the external pH, but they could not ascribe exclusively the reduction of contraction to the depression of Ca current because the extent and the time course of both were quite different.

Cadmium in Ringer's solution of normal pH caused a change in the action potential of cardiac muscle (KLEINFELD *et al.*, 1955; TODA, 1973) and of snail neuron (KOSTYUK and KRISHTAL, 1977), but the application of a low concentration of Cd ($10 \mu\text{M}$) to a frog heart for a short time (3 min) brought about a decrease in contractility without an appreciable change in the action potential (HAYASHI and HORIUCHI, 1971a). The negative inotropic effects of Cd were ascribed to interference with Ca activity which altered the excitation-contraction coupling mechanism resulting in a decrease in contractility. In this experiment, cadmium in low pH Ringer's solution caused a marked decrease in contractility without any detectable change in action potential. The effect of Cd appeared rather rapidly: time constant of decrease in contractility was about 30 sec in pH 6.5 solution (*cf.*, Fig. 1 in HORIUCHI and HAYASHI, 1978). In mammalian heart, the isolated sarcoplasmic reticulum (SR) from cardiac muscle binds Ca in low pH solution (NAKAMARU and SCHWARTZ, 1970), and thus decreases contractility. FABIATO and FABIATO (1978) pointed out that acidosis may exert the negative inotropic effect by modulating Ca sensitivity to both SR and myofilament. VAN BOGAERT *et al.* (1978) discussed the fact that alkaline solutions not only affected net negative surface charges on the outside but also, and to a greater extent, increased negative surface charges on the inside of the cell membrane. Hence, it may be possible that Cd in low pH solution may counteract Ca by competing for both these structures. However, it cannot be completely discounted that Cd in low pH solution impedes the trans-sarcolemmal Ca current which evokes the Ca-induced Ca release of SR (ENDO, 1977).

The anion of Ringer's solution might play a role in the pH dependency of the Cd effect for the following reasons: Cl permeability in barnacle muscle membrane increased as the external pH of the solution was lowered (HAGIWARA *et al.*, 1968), and the cardiac action potential was believed to be associated in part with the Cl current (HUTTER and NOBLE, 1961; LENFANT and GOUPIL, 1977). The above hypothesis was examined by substituting Cl in Ringer's solution for SO_4 which was thought to be less penetrating than Cl (HAGIWARA *et al.*, 1968; HAYASHI *et al.*, 1977). It was concluded that the hypothesis was not true because the tendency that the lowering of the pH makes Cd more effective was observed in both anion solutions. The twitch contraction of cardiac muscle was always smaller (almost half) in SO_4 -Ringer's solution than in Cl-

Ringer's solution. This may be ascribed to the difference in Ca binding to the superficial membrane corresponding to the different anions (NAYLER, 1975).

The effect of Cd on the contractility of cardiac muscle was antagonized by excess Ca. When Ca completely counteracted the Cd effect, the ratio of amount of Ca to that of Cd was 50–100 in normal pH (KLEINFELD and STEIN, 1968; HAYASHI and HORIUCHI, 1971a; TODA, 1973). This Ca/Cd ratio seemed to be different for various preparations. Cd-induced suppression of acetylcholine-release from the vagal nerve terminal was also counteracted by excess Ca, but the ratio was extremely high (1,000–2,000) (HAYASHI and TAKAYAMA, 1978). The lower the pH, the higher the amount of Ca required to counteract Cd in cardiac muscle: the Ca/Cd ratio was about 200 in pH 6.5 solution, but the ratio was only about 50 in pH 9.0 solution (*cf.*, Fig. 8). The difference in the Ca/Cd ratio may be ascribed to the difference in affinity of Cd to the Ca binding sites, but we could not identify the Ca binding site even though the ultrastructure of the frog heart was described in detail (STALEY and BENSON, 1968). REUBEN *et al.* (1971) observed that MgATP solution in the absence of Ca could elicit contraction of crayfish skinned muscle fiber. HAYASHI *et al.* (1972) found the non-competitive counteraction of ATP with Cd in cardiac muscle. This phenomenon should be investigated in more detail in relation to pH.

The amount of Cd which was really taken up by the cardiac tissue was calculated from the data of Fig. 9. Assuming the intercellular space was 30% of the frog atrium (*cf.*, HAJDU, 1953; GLITSCH, 1969) and that the concentration of Cd in the space was equal to that in the extracellular fluid, the real amount of Cd in the cardiac tissue was 4 times as much as that in the extracellular Ringer's solution (in the case of 30 μ M Cd-containing Ringer). This implied that the cardiac tissue actually took up the Cd by some mechanism not immediately obvious. The amount of Mn-uptake to the frog heart seemed to be smaller than that of Cd-uptake; Mn content in the heart was 1/20 times as much as that in the extracellular Ringer's solution (CHAPMAN and ELLIS, 1977). The higher the concentration of Cd in Ringer's solution, the more the amount of Cd taken up into the cardiac tissue. Though a marked pH dependency was observed in Cd-induced decrease in the contractility (*cf.*, Fig. 6), there was no difference in the amount of Cd-uptake whether the solution was acid or alkaline. This suggests that most of the amount of Cd-uptake is non-specific to the heart, and only a small fraction is essentially related to the Ca binding sites which are concerned with contraction. It remains to be determined what fraction or component of cardiac tissue combines most of the non-specific Cd or Cd specific to contraction.

The nature of the Ca-Cd interaction at the Ca binding sites may be inferred by the analogy of a chelating agent. The higher the stability constant of a chelating agent, the more acid the solution in which the formation of a metal chelating complex will occur. For example, the stability constant of EDTA

for Cd and Ca are 16.5 and 10.6, respectively (SAKAGUCHI and UENO, 1966); therefore, the amount of Cd-chelate complex is much larger than that of Ca-chelate complex in acid solution, which in turn may reveal the difference of divalent ion effects.

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