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The effects of K^+-depolarization medium, chelating agents and thiol groups on cadmium uptake and efflux in the guinea-pig taenia coli

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Cadmium uptake by taenia coli was dose-dependent, achieving equilibrium after approximately 60 min of incubation. The SH-blocker, *N*-ethylmaleimide inhibited cadmium uptake. When muscles were washed with normal medium or that containing 0·5 mM EDTA following 0·5 mM Cd^{2+} treatment for 60 min, the tissue cadmium concentration reached equilibrium levels after 60 min and approximately 43 or 27% of the initial tissue cadmium concentration was retained, respectively. Both glutathione and dithiothreitol also increased cadmium efflux. However the contractions of glycerinated taenia coli caused by Ca^{2+} and Mg-ATP, completely returned to control values after washing with 0·5 mM EDTA medium following 0·5 mM Cd^{2+} treatment for 60 min, suggesting that EDTA seems to exert intracellular effects in glycerinated taenia coli. These results suggest that SH-dependent mechanisms in cadmium uptake play a role in intact taenia coli. In addition, in intact taenia coli, Cd^{2+} are accumulated in intracellular compartments which EDTA cannot reach and may exert an inhibitory action on internal sites in the cells.

Previous reports have indicated that cadmium ions (Cd^{2+}) suppress contractile responses of vascular (Perry et al 1967; Thind et al 1970; Toda 1973; Nasu 1983) and intestinal (Osa 1974; Nasu et al 1983) smooth muscles to several stimulant drugs. The presumed mechanism of the action of Cd^{2+} is principally to inhibit the Ca^{2+} influx through cell membranes in both vascular (Toda 1973; Hattori et al 1983) and intestinal (Nasu et al 1983) smooth muscles. It seems possible that the quantity of the Cd^{2+} binding to the surface membrane of aorta is related to the degree of inhibition of tension (Nasu 1983). However, the antispasmodic effect of Cd^{2+} in taenia coli has been thought to be effected at various sites of action. We reported previously that muscle strips of taenia coli accumulated a far greater amount of cadmium than the extracellular space (Nasu et al 1983). Furthermore, Cd^{2+} inhibited the contraction of glycerinated taenia coli (Nasu et al 1983) and also inhibited the stage 3 respiration of isolated mitochondria (Jacobs & Jacob 1956; Sato et al 1978; Nasu et al 1983). Therefore, if Cd^{2+} penetrate the cell membrane in smooth muscle of taenia coli, they will have an effect on contractile proteins and energy metabolism. To gain further evidence of the possible site of Cd^{2+} action, the cadmium uptake and efflux in taenia coli was studied.

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MATERIALS AND METHODS

Strips of taenia coli were isolated from male Hartley strain guinea-pigs, ~400 g, and immersed in Tyrode solution bubbled with 95% O_2 and 5% CO_2 at 37 °C. The Tyrode solution contained (mM): NaCl 136·8, KCl 2·7, $CaCl_2$ 2·5, $MgCl_2$ 1·0, $NaHCO_3$ 11·9 and glucose 5·5. Phosphate-free Tyrode solution was used to avoid precipitation of cadmium phosphate. The pH of the solution was 7·3. For experiments using ethylenediaminetetraacetic acid (EDTA), a Ca^{2+}- and Mg^{2+}-free solution was prepared by omission of both $CaCl_2$ and $MgCl_2$ from the Tyrode solution. The high-K^+ solution (40 mM) was prepared by the addition of an appropriate amount of 2 M KCl solution. Cd^{2+} was added as a $CdCl_2$ solution directly to the bathing solution.

The muscles were attached to glass rods under 0·6 g resting tension and suspended to equilibrate for 60 min with several changes of solution. After equilibration, the tissue was conditioned by adding the high-K^+ solution to the bath.

To determine tissue cadmium concentration in muscles, the strips of taenia coli were removed from the bath at the end of experiments and after blotting on filter paper, they were weighed and transferred to a quartz cuvette and incinerated by a plasma asher

(Yanagimoto, Model LTA-4SN) for 10 h. As the melting point of cadmium is low (321 °C), this apparatus was suitable for incineration of the muscle. The samples were dissolved in 0·01 M HNO_3 and the Cd^{2+} concentration measured by an atomic absorption spectrophotometer (Hitachi, 207). The unknown samples were measured by comparison with known amounts of Cd^{2+} determined at the same time. The tissue cadmium concentration was expressed in terms of the wet weight of the tissue. The cadmium in taenia coli which was isolated from guinea-pigs fed with normal assorted feed was undetectable (Nasu et al 1983). By the measurement of tissue cadmium following incubation of muscles in a Cd^{2+} medium, we studied the time course of cadmium uptake and efflux.

For the tests of Cd^{2+} on contractile proteins, the muscles were glycerinated. Strips of taenia coli were placed in a solution which contained 50% (v/v) glycerin, 50 mM KCl, 20 mM Tris-malate (pH 6·8) and 0·2 mM Mg-ATP and were stored at 2 °C. After 24 h the strips were replaced in a fresh glycerin solution and stored at −15 °C for 6 days. In experiments, the glycerinated muscles were incubated in a 'relaxing solution' containing 50 mM KCl, 20 mM Tris-malate (pH 6·8) and 0·2 mM Mg-ATP. Because the Cd^{2+} binding potency of ethylene glycol bis(β-aminoethylether) tetraacetic acid (EGTA) is higher than its Ca^{2+} binding potency, the EGTA-Ca EGTA buffer system could not be used to make a medium giving a low stabilized concentration free ionized calcium. Therefore, a solution containing a high concentration of Ca^{2+} (0·1 mM, no EDTA) and 5 mM Mg-ATP was used to produce contractions. The contractile responses were recorded isotonically with a magnification of 7 : 1 under a resting tension of 100 mg at 24 °C.

RESULTS

Strips of taenia coli were incubated in a medium containing different Cd^{2+} concentrations (0·06, 0·1, 0·2 or 0·5 mM). The tissue cadmium concentration in each medium was plotted against the incubation time (Fig. 1). The cadmium uptake reached an equilibrium which was dependent on the Cd^{2+} concentration. The time required to reach equilibrium levels was almost the same (approximately 60 min) for each Cd^{2+} concentration. The tissue/medium (T/M) Cd^{2+} concentration ratios at equilibrium levels were 8·5, 6·9, 5·7 and 3·5 in the presence of 0·06, 0·1, 0·2 and 0·5 mM Cd^{2+} in the medium, respectively.

The cadmium uptake and efflux in taenia coli in 0·5 mM Cd^{2+} medium was chiefly studied, because it completely inhibited the high-K^+ (40 mM)-induced contraction of taenia coli within 3–5 min (Nasu et al 1983).

The effect of high-K^+ (40 mM) on cadmium uptake was shown in Fig. 2. Muscles were incubated in a

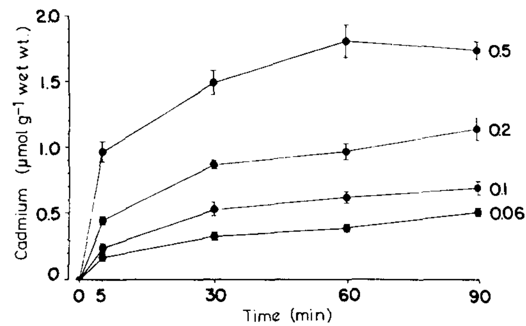

FIG. 1. Time course of cadmium uptake by taenia coli. $CdCl_2$ was added at time 0. The accompanying number for each curve indicates the concentration of Cd^{2+} in the medium (mM). Ordinate: Tissue cadmium concentration μmol g^{-1} wet wt). Abscissa: Time (min). Each point in this figure represents the mean of 16–20 experiments (mean ± s.e.).

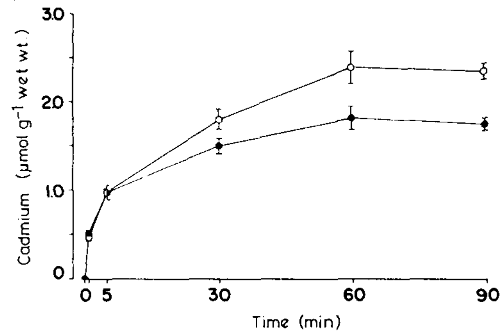

FIG. 2. Cadmium uptake of taenia coli in high-K^+ (40 mM) medium. After the incubation in high-K^+ medium for 30 min, the muscles were incubated in high-K^+ medium containing 0·5 mM Cd^{2+}. $CdCl_2$ was added at time 0. Each point in this figure represents the mean of 6–12 experiments. High-K^+ caused a significant increase in cadmium uptake at 30, 60 and 90 min after the application of $CdCl_2$. ●, 0·5 mM Cd^{2+}; ○, High-K^+ + 0·5 mM Cd^{2+}.

Table 1. Effects of *N*-ethylmaleimide (NEM) on cadmium uptake by taenia coli. Muscles were preincubated in 1 mM NEM for 30 min and then washed three times with normal Tyrode solution. The uptake of 0·5 mM Cd^{2+} for 5, 30 and 60 min incubation was determined. Results are expressed as mean ± s.e. (n = 6–10).

	Cadmium uptake (μmol g^{-1} wet weight tissue)		
	5 min	30 min	60 min
Control	0·97 ± 0·08	1·50 ± 0·09	1·82 ± 0·13
1 mM NEM	0·72 ± 0·06*	1·12 ± 0·08*	1·35 ± 0·09*

* Indicate the values significantly different ($P < 0·05$) from control values.

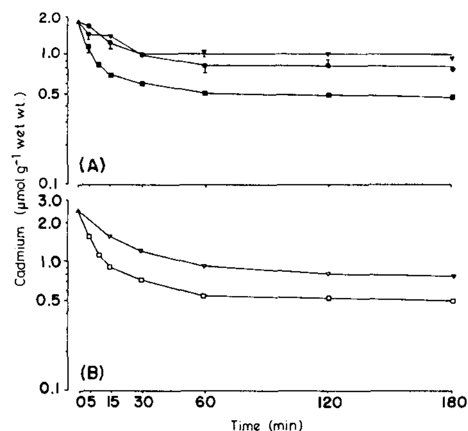

FIG. 3. Cadmium efflux from taenia coli. (A) Muscles preincubated with 0·5 mM Cd^{2+} in normal medium (▲) for 60 min were washed subsequently with normal medium (●), Ca^{2+}- and Mg^{2+}-free medium (▼) or Ca^{2+}- and Mg^{2+}-free medium containing 0·5 mM EDTA (■). (B) Muscles preincubated with 0·5 mM Cd^{2+} in high-K^+ medium (△) for 60 min were washed subsequently with Ca^{2+}- and Mg^{2+}-free, high-K^+ medium (▽) or Ca^{2+}- and Mg^{2+}-free, high-K^+ medium containing 0·5 M EDTA (□). Ordinate scale: Tissue cadmium concentration (μmol g^{-1} wet wt), logarithmic scale. Abscissa scale: Time (min). Each point represents the mean of 10–15 experiments. Vertical bars indicate the standard error. In the absence of a bar, standard errors were less than the size of the symbol.

high-K^+ medium containing 0·5 mM Cd^{2+}. The rate of cadmium uptake was not affected in the early phase, however, it increased significantly from control levels in the late phase.

Pretreatment with sulphydryl groups (SH) blocker. 1 mM *N*-ethylmaleimide (NEM) significantly decreased the cadmium uptake (Table 1).

The changes in cadmium efflux were studied. Strips of taenia coli were incubated in 0·5 mM Cd^{2+} medium for 60 min and subsequently washed with normal medium. The cadmium efflux curve could be divided into a fast and slow component. The cadmium content of the muscles reached equilibrium levels after 60 min in normal medium and about 43% of the original tissue cadmium was retained. When the muscle strips were washed with a Ca^{2+}- and Mg^{2+}-free medium, the cadmium remaining in the muscles did not differ from the control values (Fig. 3A).

Previous experiments have indicated that the chelating agent, EDTA, does not penetrate the cell membrane of guinea-pig taenia coli (Brading & Jones 1969). In addition, the action of EDTA appears to be restricted to the smooth muscle cell membrane compartment (Weiss & Goodman 1976). The effects of EDTA on cadmium retention in muscles were investigated. The muscles were incubated in a 0·5 mM Cd^{2+} medium for 60 min and subsequently washed with Ca^{2+}- and Mg^{2+}-free medium containing 0·5 mM EDTA (Fig. 3A). The cadmium content of the muscles reached equilibrium levels approximately 60 min after the washing and about 27% of the initial tissue cadmium content was retained after 60 min washing with the medium containing 0·5 mM EDTA.

The effects of high-K^+ on cadmium efflux were also studied. Muscles preincubated with high-K^+ medium containing 0·5 mM Cd^{2+} for 60 min were washed subsequently with a Ca^{2+}- and Mg^{2+}-free, high-K^+ medium containing 0·5 mM EDTA. A similar concentration of cadmium retention in the muscles was obtained after washing with the high-K^+, EDTA medium at equilibrium, compared with washing with normal K^+, EDTA medium (Fig. 3B). When the muscles were washed with medium containing 5 mM EDTA and subjected to the same experimental design, the results did not differ significantly.

Effects of thiol agents on cadmium efflux from taenia coli were examined. Strips of taenia coli were incubated in 0·5 mM Cd^{2+} medium for 60 min and subsequently washed with the solution containing 1 mM glutathione or 1 mM dithiothreitol. These thiol agents increased the cadmium efflux (Table 2).

Table 2. Effects of thiol agents on cadmium efflux from taenia coli. Muscles were incubated with 0·5 mM Cd^{2+} for 60 min before washing. The muscles were washed with solution containing 1 mM glutathione or 1 mM dithiothreitol. Results are expressed as mean ± s.e. (n = 10–12).

	Cadmium efflux (μmol g^{-1} wet weight tissue)			
	5 min	15 min	30 min	60 min
Control	1·70 ± 0·06	1·25 ± 0·12	0·99 ± 0·07	0·83 ± 0·08
Glutathione	1·44 ± 0·06*	0·98 ± 0·10*	0·80 ± 0·04*	0·65 ± 0·06*
Dithiothreitol	1·47 ± 0·07*	0·93 ± 0·10*	0·71 ± 0·05*	0·63 ± 0·04*

* Indicate the values significantly different ($P < 0·05$) from control values.

A schematic diagram of cadmium distribution in taenia coli is shown in Fig. 4. The total cadmium spaces were obtained from the tissue cadmium concentration when muscle strips were incubated with normal or high-K^+ medium containing 0·5 mM Cd^{2+} for 60 min. On the assumption that extracellular space is completely saturated with 0·5 mM Cd^{2+} equal to the external bathing medium, the hatched area of the figure represents the amount of Cd^{2+} concentration in the extracellular space [0·5 mM Cd^{2+} in the external medium × 0·35 in extracellular space measured by [^{14}C]sorbitol (Nasu et al 1983)]. The cadmium fraction in the cells was divided into three parts: 'A fraction': the cadmium fraction that

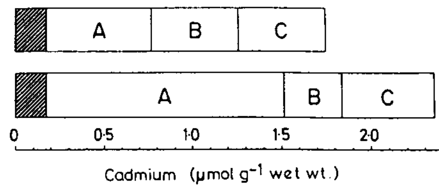

FIG. 4. A schematic diagram of cadmium distribution in taenia coli. Total cadmium spaces were obtained from tissue cadmium concentration following 0·5 mM Cd^{2+} treatment in normal or high-K^+ (40 mM) medium for 60 min. 'A': Cadmium fraction that was released in Cd^{2+}-free medium for 60 min. 'B': Cadmium fraction that was eliminated after washing with 0·5 mM EDTA medium for 60 min. 'C': Cadmium fraction that was not eliminated by EDTA. The muscles preincubated with high-K^+ medium containing 0·5 mM Cd^{2+} were washed with Cd^{2+}-free, high-K^+ medium. Assuming that extracellular space is completely saturated with 0·5 mM Cd^{2+} equal to the external bathing fluid, the hatched area of the figure represents the amount of Cd^{2+} concentration in the extracellular space. Each fraction was calculated from experimental data. Upper panel: Cadmium distribution of taenia coli in 0·5 mM Cd^{2+} medium. Lower panel: Cadmium distribution of taenia coli in high-K^+ (40 mM) medium containing 0·5 mM Cd^{2+}.

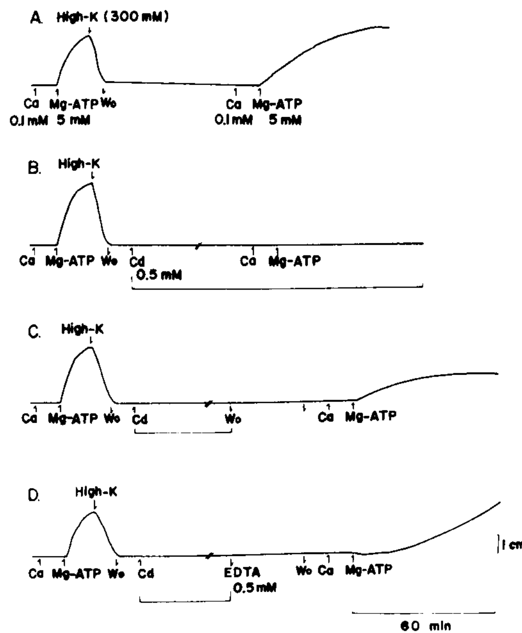

FIG. 5. Effects of washing on recovery of contraction after Cd^{2+} treatment in glycerinated taenia coli. (A) Shortening to 0·1 mM Ca^{2+} and 5 mM Mg-ATP. (B) Shortening of glycerinated taenia coli in the presence of 0·5 mM Cd^{2+}. After treatment with 0·5 mM Cd^{2+} for 60 min, the muscles were washed with 'relaxing solution' (50 mM KCl, 20 mM Tris-malate (pH 6·8) and 0·2 mM Mg-ATP) (C) or 'relaxing solution' containing 0·5 mM EDTA for 30 min (D). After returning to fresh 'relaxing solution', 0·1 M Ca^{2+} and 5 mM Mg-ATP was applied to the glycerinated taenia coli.

was released after washing with Cd^{2+}-free medium for 60 min, 'B fraction': the cadmium fraction that was eliminated after washing with EDTA medium for 60 min, and 'C fraction': the cadmium fraction that was not eliminated by EDTA.

The glycerinated taenia coli contracted in response to 0·1 mM Ca^{2+} and 5 mM Mg-ATP and took 15–20 min to reach a maximum (Fig. 5A). Briggs (1963) demonstrated that K^+ concentration above 50 mM increased the rate of relaxation of glycerinated uterine strips, presumably relating to dissociation of the contractile proteins following contraction as the ionic strength is increased. The glycerinated taenia coli fully relaxed in the 'relaxing solution' (50 mM KCl, 20 mM Tris-malate (pH 6·8) and 0·2 mM Mg-ATP) with added high concentrations of K^+ (300 mM). This shortening was reversible, however, the rate of second shortening was slower (Fig. 5A). A complete inhibition of the shortening occurred in the presence of 0·5 mM Cd^{2+} (Fig. 5B). To test whether washing has any effect on recovery of contraction after Cd^{2+} treatment, glycerinated taenia coli were first incubated in 0·5 mM Cd^{2+} for 60 min and then washed with 'relaxing solution' or the solution containing 0·5 mM EDTA for 30 min. After returning to fresh 'relaxing solution', 0·1 mM Ca^{2+} and Mg-ATP was applied. Responses to Ca^{2+} and Mg-ATP were 53 ± 7% (n = 10) of the controls after washing with 'relaxing solution' (Fig. 5C). In comparison, the response returned to control values after washing with EDTA solution (Fig. 5D).

DISCUSSION

The present experiment demonstrates that the cadmium uptake into taenia coli was dose- and time-dependent, achieving equilibrium after 60 min of incubation. Schnieden & Small (1971) showed by using radioactive ^{115}Cd that Cd^{2+} have very much larger apparent volumes of distribution than the extracellular marker, sorbitol, in the ileal longitudinal strips. They assumed that there is surface binding to cell membrane and/or intracellular accumulation of Cd^{2+}. We have further studied the cadmium uptake and efflux in an attempt to define the property of Cd^{2+} binding to taenia coli.

The schematic diagram of cadmium distribution in taenia coli is shown in Fig. 4. The 'A fraction' means cadmium of the cells that was excluded by Cd^{2+}-free medium following 0·5 mM Cd^{2+} treatment. It is considered that cadmium of the 'A fraction' is loosely bound to the cell membrane. Figs 3 and 4 indicate that the increase in the total tissue cadmium concentration of taenia coli in high-K^+ medium

results from the increased 'A fraction'. The 'A fraction' in taenia coli after Cd^{2+} treatment also increased at low temperature (4 °C) (Nasu & Koshiba 1984a). It has been reported (Magaribuchi et al 1973) that membranes of taenia coli depolarized when the temperature was lowered from 32 to 10 °C. These facts may indicate that the increase of 'A fraction' in high-K^+ medium is related to membrane depolarization.

The biochemical basis of effects of heavy metals are generally thought to involve heavy metal-sulphur interaction (Vallee & Ulmer 1972; Toda 1973). The interaction of heavy metals with albumin or cysteine has served as model for studies of heavy metal binding by proteins in general. Heavy metal as Hg^{2+} binds strongly to –SH and S–S groups in proteins although the binding is reversible (Burstein & Sperling 1970; Vallee & Ulmer 1972). In the present study, the SH blocker, NEM, inhibited cadmium uptake in taenia coli. We have further found that thiol agents (glutathione, dithiothreitol) increased cadmium efflux in taenia coli. This suggests that a SH-dependent mechanism plays a role in cadmium uptake and Cd^{2+} can bind to SH groups on the cell membrane of taenia coli.

While EDTA has a remarkable affinity for Cd^{2+} (log of equilibrium constant = 16·46, Anderegg 1964), cysteine-containing sulphydryl groups have a lesser affinity (log of equilibrium constant = 9·89, Suffet & Purdy 1966) than EDTA. Therefore, the cadmium from the S–Cd–S bond will be eliminated by EDTA and so a part of the 'B fraction' in taenia coli may contain cadmium which was bound to SH groups on the surface membrane. Cd^{2+} also inhibited the high-K^+-induced ^{45}Ca uptake in taenia coli (Nasu et al 1983). Presumably cadmium in the 'B fraction' may compete with Ca^{2+} at the cell membrane of taenia coli.

In aorta, the high-K^+-induced contraction completely returned to control values after washing with EDTA medium, following a treatment with 0·5 mM Cd^{2+} for 30 min. EDTA increased Cd^{2+} efflux and only 5% of the initial tissue cadmium content was retained after a 60 min wash in EDTA medium (Nasu 1983). It is suggested that Cd^{2+} binds chiefly to the surface membrane of aorta. However, following a 30 min incubation with 0·5 mM Cd^{2+}, the response of taenia coli to high-K^+ had not returned in spite of the washing with EDTA medium and the 'C fraction', which was not eliminated by EDTA, increased (Nasu & Koshiba 1984b). We have further found that the increase in the 'C fraction' following Cd^{2+} treatment is temperature dependent; the 'C fraction' was small after 60 min treatment with 0·5 mM Cd^{2+} at low temperatures (4 °C) in taenia coli (Nasu & Koshiba 1984a; Nasu 1984).

From these facts, the increase in the 'C fraction' following Cd^{2+} treatment could be explained by the fact that Cd^{2+} are accumulated in the intracellular compartment where EDTA cannot reach. However, there remains the possibility that the inclusion of EDTA in the bathing fluid facilitates the Cd^{2+} loss from the intracellular compartment, presumably by displacing bound Cd^{2+} from the cell membrane. An effect of EDTA would be to decrease the size of 'C fraction'. However, the maintenance of the retention of tissue cadmium at the equilibrium levels after the inclusion of EDTA for longer times (180 min) (Fig. 3) indicates that the facilitating effect of Cd^{2+} loss from tissue by EDTA would be small.

It has been suggested that the main site of action of the non-penetrating chelator, EDTA (Brading & Jones 1969) is on the cell membrane of intact smooth muscle (Weiss & Goodman 1976). The suggestion is supported by our experiments with glycerinated taenia coli. The contractions of glycerinated taenia coli to Ca^{2+} and Mg-ATP returned to control values after washing with EDTA medium following longer incubation periods (60 min) with 0·5 mM Cd^{2+} (Fig. 5D). Since all molecules in the external medium can penetrate the glycerinated taenia coli, the EDTA would directly affect the contractile proteins. On the other hand, the response of glycerinated taenia coli returned to about 50% of control values after treatment with 0·5 mM Cd^{2+} for 60 min (Fig. 5C), suggesting that Cd^{2+} effects on contractile proteins were easily washed out.

Finally, the inhibitory action of Cd^{2+} in taenia coli is mainly thought to be attributable to decreased availability of Ca^{2+} (Nasu et al 1983). Furthermore, Cd^{2+} may exert secondary actions on internal sites of cells, such as mitochondria and contractile proteins.

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