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Effect of cadmium on transmembrane Na^+ and K^+ transport systems in human erythrocytes

P Lijnen, J Staessen, R Fagard, A Amery

Abstract

The effects of cadmium (Cd^{2+}) on Na^+, K^+-ATPase in disrupted human erythrocyte membranes and on various transmembrane Na^+ and K^+ transport systems in intact erythrocyte suspensions were studied. Cadmium^{2+} inhibited the erythrocyte Na^+, K^+-ATPase enzyme with a 50% inhibition at a Cd^{2+} concentration of 6·25 μM. The Cd^{2+} inhibition in the human erythrocyte was non-competitive with respect to Na^+, K^+, and ATP. Cadmium^{2+} exerted no acute effect, however, on the Na^+, K^+-ATPase pump activity as measured by the ouabain sensitive ^{86}Rb uptake or Na^+ efflux in intact red blood cells. Cadmium^{2+} also inhibited the Ca^{2+} dependent K^+ channels in human red blood cells, whereas it had no effect on Na^+, K^+ cotransport, Na^+, Li^+ countertransport, anion carrier, and the number of active Na^+ pump units. The data indicate that in human erythrocytes under acute conditions Cd^{2+} exerts an inhibitory effect on Na^+, K^+-ATPase enzyme in disrupted erythrocytes and the Ca^{2+} stimulated K^+ efflux in intact red blood cells without affecting the Na^+ pump, Na^+, K^+ cotransport, and Na^+, Li^+ countertransport activity.

Cadmium (Cd^{2+}) has been reported to inhibit Na^+, K^+-ATPase in homogenates and subcellular fractions in a variety of organs and tissues originating from different animal species.[1-5] Vascular smooth muscle cells (VSMCs) derived from the rat carotid artery have been cultured and studied in vitro; Cd^{2+} inhibited Na^+, K^+-ATPase in disrupted VSMCs but had no effect on the Na^+, K^+ pump in intact VSMCs.[6]

In the present study we have investigated the effect of Cd^{2+} on Na^+, K^+-ATPase in disrupted human erythrocytes. Also, suspensions of intact human red blood cells were used to study the influence of Cd^{2+} on various transmembrane Na^+, K^+ transport systems, such as Na^+, K^+-ATPase pump, Na^+, K^+ cotransport, Na^+, Li^+ countertransport, anion carrier, number of active Na^+ pump units, and Ca^{2+} stimulated K^+ efflux.

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Methods

Venous blood was withdrawn from five normal non-smoking men into heparinised tubes. The plasma and buffy coat were removed and the fresh red blood cells were separated and prepared as described previously.[7] Intra-erythrocyte Na^+ and K^+ concentration was determined by atomic absorption spectrometry.[7] The activity of the erythrocyte Na^+, K^+-ATPase pump was measured by the ouabain induced inhibition of uptake of ^{86}Rb in a Ringer medium with 4 mM K^+ during a 15 minute incubation at 37°C.[8] The erythrocyte bumetanide sensitive ^{86}Rb uptake reflects the influx of K^+ through the cotransport system and the bumetanide, ouabain-resistant ^{86}Rb uptake the passive permeability of the cell membrane.[9] The maximal ^3H-ouabain binding of erythrocytes, an estimate of the number of active Na^+ pump units, was assayed according to the technique of De Luise and Flier.[10]

The ouabain sensitive Na^+ efflux was measured as half the increase in erythrocyte Na content during incubation of whole blood with 0·1 mM ouabain at 37°C for two hours.[11] The DIDS (4,4'-diisothiocyanostilbene-2,2'-disulphonate) sensitive $LiCO_3^-$ influx in erythrocytes incubated in a CO_3^{2-} medium was taken as a measure of $LiCO_3^-$ influx through the anion carrier.[12] The latter catalyses a $Cl^-/NaCO_3^-$ or $HCO_3^-/NaCO_3^-$ exchange.

The erythrocyte Ca^{2+} dependent K^+ channels were measured as the K^+ efflux stimulated by the calcium ionophore A23187 (0·15 μM) or calimycin.[13,14]

The red cell Na^+, Li^+ countertransport activity or sodium stimulated Li^+ efflux, which can be considered as a measure of the Na^+, H^+ exchange,[15] was calculated by subtracting the Li^+ efflux assayed in Li^+ loaded red blood cells in the Na^+ free medium from that in the Na^+ enriched medium.[16]

Erythrocyte ghost Na^+, K^+-ATPase was isolated and assayed as the release of inorganic phosphate (Pi) from ATP after a 30 minute incubation at 37°C in a medium containing (final concentrations) 100 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 5 mM ATP, 0·1 mM ethylenediaminetetra-acetic acid (EDTA), and

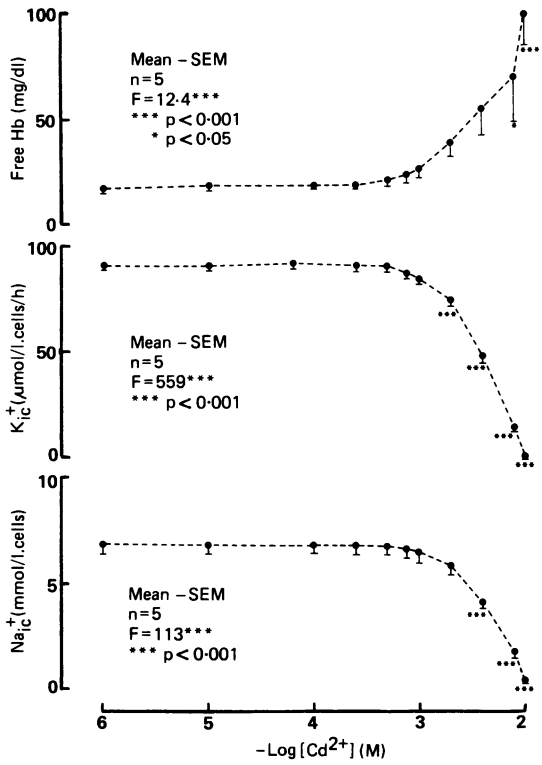

Figure 1 Effect of Cd^{2+} (1 nM to 10 mM) on the free haemoglobin (Hb) concentration in the supernatant and on the intracellular (ic) K^+ and Na^+ concentration of red blood cells incubated at 37°C for one hour at a haematocrit of 5% in a Na^+ free medium containing 75 mM $MgCl_2$, 85 mM sucrose, 10 mM glucose, 0·1 mM ouabain and 10 mM MOPS-Tris (at a pH of 7·4 at 37°C).

30 mM imidazole (pH 7·4) and in a medium containing 0·1 mM ouabain but no KCl.[17]

The presence of free haemoglobin in the supernatants of the erythrocyte suspensions before and after various incubation times, assayed spectrophotometrically after oxidation of diphenylamine in the presence of hydrogen peroxide,[18] was taken as an indicator of possible haemolysis of the erythrocytes.

Cadmium^{2+} (in the form of pure $CdCl_2$) in a concentration range of 1 nM to 1 mM was added to the various incubation media of the above mentioned flux assays.

Before use all the laboratory tubes were thoroughly cleaned with a brush and detergent. Thereafter they were filled with 65% of nitric acid and deionised water up to their total volume and left for 24 hours. They were then rinsed five times consecutively with deionised water and dried in an oven. Before use in the study a random sample of the prepared tubes and of the solvents used in the various procedures were checked for Cd contamination and none was found to be positive.

The statistical methods used were two way analysis of variance with Scheffe's multiple mean test. Subjects and time were considered as sources of variation. The dispersion of the data is given by standard error of the mean.

Results

HAEMOLYSIS OF ERYTHROCYTES BY HIGH CONCENTRATIONS OF Cd^{2+}

Incubation of erythrocytes with Cd^{2+} in a concentration range from 1 nM to 10 mM for one hour at 37°C showed that concentrations above 1 mM Cd^{2+} all caused haemolysis that was greater than 1% (fig 1).

Table 1 Effect of Cd^{2+} (10 nM to 1 mM) on ouabain sensitive ^{86}Rb uptake and Na^+ efflux; bumetanide sensitive ^{86}Rb uptake; ouabain, bumetanide resistant ^{86}Rb uptake; Na^+, Li^+ countertransport; and maximal ^3H-ouabain binding and anion carrier in human erythrocytes

	Concentration of Cd^{2+}							
	0	*10 nM*	*100 nM*	*1 μM*	*10 μM*	*100 μM*	*1 mM*	*F Value*
OS ^{86}Rb uptake (μmol/l.cells/h)	1·99±0·07	1·91±0·06	1·94±0·07	1·95±0·05	1·93±0·07	1·94±0·07	1·97±0·07	1·98^{NS}
OS Na^+ efflux (mmol/l.cells/h)	1·90±0·06	1·85±0·03	1·85±0·02	1·83±0·03	1·78±0·05	1·76±0·03	1·85±0·03	1·71^{NS}
BS ^{86}Rb uptake (μmol/l.cells/h)	0·47±0·08	0·46±0·09	0·45±0·09	0·46±0·08	0·47±0·09	0·47±0·09	0·43±0·08†	3·38*
OBR ^{86}Rb uptake (μmol/l.cells/h)	0·106±0·006	0·104±0·005	0·108±0·007	0·102±0·002	0·104±0·002	0·106±0·005	0·116±0·002	1·80^{NS}
Na^+, Li^+ countertransport (μmol/l.cells/h)	407±33	409±33	410±33	410±34	416±37	432±35	384±32	1·11^{NS}
Maximal ^3H-ouabain binding (nmol/l.cells/h)	4·34±0·39	4·34±0·38	4·34±0·38	4·34±0·40	4·33±0·39	4·48±0·39	3·79±0·32**	9·35***
Anion carrier (μmol/l.cells/h)	77·4±2·8	78·2±3·3	74·4±1·9	77·2±2·2	79·6±3·8	76·5±2·6	56·3±1·1***	36·5***

Values are means ± SEM (n = 5).
OS = Ouabain sensitive; BS = bumetanide sensitive; OBR = ouabain, bumetanide resistant.
*p < 0·05; **p < 0·01; ***p < 0·001; NS = non-significant.
†p = 0·06.

Table 2 Effect of preincubation of red blood cells or whole blood with and without 100 μM Cd^{2+} for various times on ouabain-sensitive ^{86}Rb uptake or Na^+ efflux

		Preincubation time (min)				
		0	30	60	120	240
Red blood cells:						
OS ^{86}Rb uptake (μmol/l.cells/h)	No Cd^{2+}	1·87±0·04	1·87±0·04	1·84±0·03	1·81±0·04	1·81±0·04
	100 μM Cd^{2+}	1·88±0·05	1·89±0·05	1·85±0·07	1·77±0·05	1·72±0·07
Whole blood:						
OS Na^+ efflux (mmol/l.cells/h)	No Cd^{2+}	2·10±0·05	2·10±0·05	2·09±0·04	1·65±0·06	1·51±0·05
	100 μM Cd^{2+}	2·09±0·05	2·10±0·05	2·09±0·05	1·68±0·05	1·53±0·04

Values are means ± SEM (n = 5).

Haemolysis above 1 mM Cd^{2+} was accompanied by a decrease in the intracellular K^+ and Na^+ concentration. In view of the 1% haemolysis seen above 1 mM Cd^{2+} the effect of Cd^{2+} on the erythrocyte transmembrane Na^+, K^+ transport systems was only investigated in the concentration range from 1 nM to 1 mM. In this concentration range the intracellular K^+ concentration was stable at 90% of the control value of 90·9 ± 2·4 mmol/l cells.

EFFECT OF Cd^{2+} ON THE Na^+, K^+ TRANSPORT SYSTEMS IN ERYTHROCYTES IN SUSPENSION

As shown in table 1 no effect of Cd^{2+} in the concentration range of 10 nM to 1 mM was found on the erythrocyte ouabain sensitive ^{86}Rb uptake or Na^+ efflux, ouabain, bumetanide resistant ^{86}Rb uptake, and Na^+, Li^+ countertransport activity.

The bumetanide sensitive ^{86}Rb uptake tended to decrease ($p = 0·06$) while the maximal ^3H-ouabain binding and the anion carrier were significantly decreased at a Cd^{2+} concentration of 1 mM. At lower Cd^{2+} concentration no effect was seen on the erythrocyte bumetanide sensitive ^{86}Rb uptake, anion carrier, and maximal ^3H-ouabain binding.

Preincubation of erythrocytes or whole blood with 100 μM Cd^{2+} at 37°C for 0·5, one, two, and four hours before the assay of the ouabain sensitive ^{86}Rb uptake or Na^+ efflux did not affect the Na^+, K^+-ATPase pump activity in these intact erythrocytes (table 2).

EFFECT OF Cd^{2+} ON ERYTHROCYTE MEMBRANE Na^+, K^+-ATPase

The specific activity of Na^+, K^+-ATPase in disrupted erythrocyte membranes was 0·48 ± 0·02 μmol Pi/mg protein/h.

In preliminary experiments no effect of different concentrations of Cd^{2+} was seen on the activity of Na^+, K^+-ATPase when it was mixed in the substrate solutions used to assay the enzyme, probably because of binding of Cd^{2+} to imidazole that was used as the buffer system in the substrate solutions. The inhibition of Cd^{2+} on erythrocyte Na^+, K^+-ATPase could be shown when the red blood cell membrane suspensions were preincubated with Cd^{2+} at 37°C for 30 minutes before the enzymatic assays.

Figure 2 depicts the dose response curve for Cd^{2+} inhibition of the erythrocyte Na^+, K^+-ATPase. The I_{50} (50% inhibition) by Cd^{2+} was reached at concentrations of 6·25 μM and almost complete inhibition of the enzyme was obtained at 100 μM.

Enzyme kinetic analysis showed that inhibition of the erythrocyte Na^+, K^+-ATPase by Cd^{2+} at a concentration of 6·25 μM resulted from a reduction of the maximal rate of the enzymatic reaction rather than from an alteration in the apparent K_m (criterion for affinity) of the enzyme to K^+, Na^+, and ATP (fig 3). Indeed, the affinities of Na^+, K^+-ATPase for Na^+, K^+, and ATP determined from Lineweaver-Burk plots of the inverse rate (1/v) *v* the inverse ligand concentration, were not different in the presence or absence of 6·25 μM Cd^{2+} (table 3).

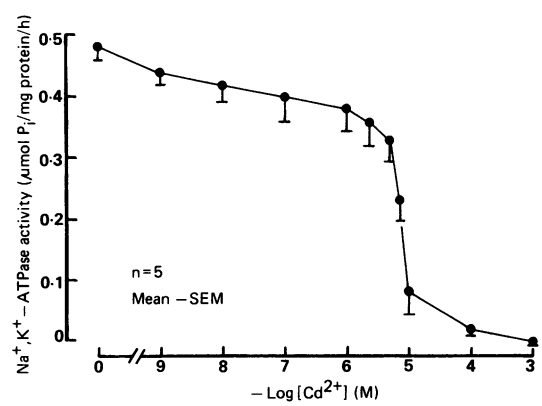

Figure 2 Dose response curve for the effect of Cd^{2+} on the erythrocyte Na^+, K^+-ATPase. Before the enzymatic assays the red blood cell membrane suspensions were preincubated in the absence or presence of Cd^{2+} at 37°C for 30 minutes.

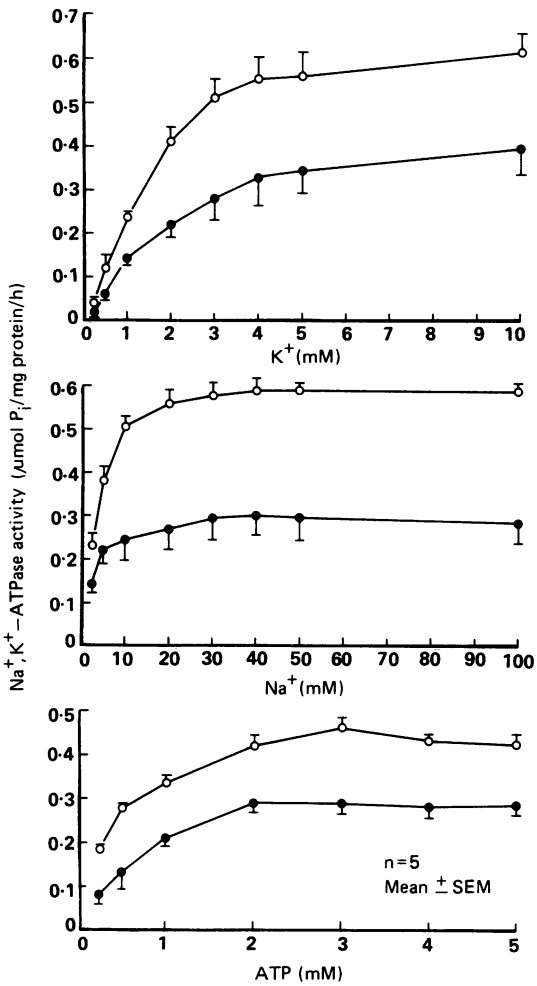

Figure 3 Potassium, Na^+, and ATP kinetics of Na^+, K^+-ATPase under control conditions (○) and in the presence of 6·25 μM Cd^{2+} (●).

EFFECT OF Cd^{2+} ON ERYTHROCYTE Ca^{2+} DEPENDENT K^+ CHANNELS

Cd^{2+} inhibited the Ca^{2+} stimulated K^+ efflux in erythrocytes dose dependently (fig 4) with an I_{50} at a concentration of 18 μM. Figure 5 shows the effect of the Ca ionophore calimycin in a concentration range of 0·1 to 2 μM on the erythrocyte Ca^{2+} stimulated K^+ efflux in the presence or absence of 18 μM Cd^{2+}. The % inhibition of the Ca^{2+} stimulated K^+ efflux decreased progressively with higher concentrations of calimycin.

The internal Ca^{2+} concentrations in the erythrocytes were increased by raising artificially the effective Ca^{2+} permeability of the cell membrane with the use of 1 μM calimycin (fig 6). With this procedure the intra-erythrocyte Ca^{2+} concentration was increased to 4·32 ± 0·73 μmol/l cells after 10 minutes incubation but only to 3·11 ± 0·71 μmol/l cells in the presence of 18 μM Cd^{2+}. Further incubation of the erythrocytes with 1 μM calimycin for 30 minutes induced a further rise in intracellular Ca^{2+} concentration to 5·76 ± 0·72 μmol/l cells. Addition of 2 mM ethylene glycol tetra-acetic acid (EGTA) induced a fall in the intracellular Ca^{2+} concentration in the presence as well as in the absence of 18 μM Cd^{2+}.

Discussion

The present study shows that the human erythrocyte Na^+, K^+-ATPase enzyme is sensitive to Cd^{2+} inhibition. The I_{50} for Cd^{2+} inhibition of the enzyme in our experiments was reached at 6·25 μM. Other investigators reported I_{50} values of approximately 1 μM for Cd^{2+} inhibition of Na^+, K^+-ATPase in microsomal preparations of the rat kidney and rat brain synaptosomes[3 5] and of 10 μM for Cd^{2+} inhibition of VSMC Na^+, K^+-ATPase derived from rat carotid artery.[6] Na^+, K^+-ATPase preparations derived from human or canine kidneys are more resistant to Cd^{2+} inhibition with reported I_{50} values being attained at 100 μM.[1 19 20] It is well recognised that toxic metal ions such as Cd^{2+}, Pb^{2+}, Hg^{2+}, Mn^{2+}, Al^{3+}, etc inhibit Na^+, K^+-ATPase in homogenates and subcellular fractions derived from a variety of tissues.[1-3 21-23]

The inhibitory action of Cd^{2+} on the erythrocyte Na^+, K^+-ATPase enzyme is probably not specific to this enzyme system. Indeed, divalent heavy metals exert their toxicity at the cellular level by several mechanisms which ultimately alter the activity of various enzymes.[24]

As the Na^+, K^+-ATPase enzyme is rich in thiol groups and sulphydryl reagents inhibit the enzyme,[25 26] it is likely that Cd^{2+} and other heavy metals inhibit the Na^+, K^+-ATPase in a variety of tissues including human red blood cells by its high affinity for these thiol residues in the cell membrane. Tokushige *et al*[6] have shown that other divalent metals such as Pb^{2+} and Hg^{2+} also inhibit the Na^+, K^+-ATPase in cultured VSMCs of the rat carotid artery.

The inhibitory action of Cd^{2+} on the erythrocyte

Table 3 Affinities of Na^+, K^+-ATPase for Na^+, K^+, and ATP

	K_m (mM)	
	without Cd^{2+}	*with 6·25 μM Cd^{2+}*
Na^+	8·21 ± 0·06	8·30 ± 0·09
K^+	1·68 ± 0·03	1·70 ± 0·02
ATP	0·33 ± 0·01	0·36 ± 0·01

Values are means ± SEM (n = 5).

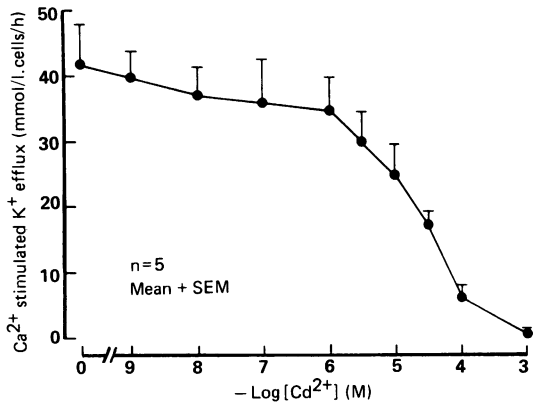

Figure 4 Dose response curve for the effect of Cd^{2+} on the erythrocyte Ca^{2+} stimulated K^+ efflux. The intact red blood cells were incubated at 37°C for 10 minutes at a haematocrit of 0·5% in a Cs^+/Na^+ medium containing 140 mM NaCl, 10 mM CsCl, 1 mM $CaCl_2$, 10 mM MOPS-Tris (pH 7·4 at 37°C), 0·1 mM ouabain, 0·02 mM bumetanide, and 10 mM glucose in the presence of 0·15 μM of the ionophore AH23187 or calimycin.

Na^+, K^+-ATPase enzyme is probably mediated through toxic damage to the enzyme units as evidenced by the following observations. Firstly, enzyme kinetic analysis (fig 3) shows that the inhibitory effect of Cd^{2+} on erythrocyte Na^+, K^+-ATPase was non-competitive with respect to Na^+, K^+, and ATP and resulted from a reduction in the maximal rate (V_{max}) of the enzymatic reaction rather than from an alteration of the affinity (K_m) of the enzyme for its substrate. Secondly, a chelator such as EDTA was ineffective in reversing the Cd^{2+} inhibition of VSMC Na^+, K^+-ATPase.[6] In disrupted VSMCs, reversal of Cd^{2+}-inhibition by EDTA would have been expected if Cd^{2+} had not exerted irreversible toxic effects on the Na^+, K^+-ATPase system.[6]

Despite a profound inhibition of the Na^+, K^+-ATPase system in disrupted erythrocyte membranes, no effect of Cd^{2+} was found on the activity of the Na^+, K^+-ATPase pump as measured by the ouabain sensitive ^{86}Rb uptake or Na^+ efflux in suspensions of intact red blood cells (tables 1 and 2). Longer exposure, up to four hours, of the red blood cells or whole blood with Cd^{2+} did not induce an effect on the Na^+, K^+-ATPase pump activity (table 2). Tokushige *et al*[6] also reported an inhibition of VSMC Na^+, K^+-ATPase by Cd^{2+} without any effect on the ouabain sensitive ^{86}Rb uptake by the cultured VSMCs although Cd^{2+} was taken up by the cells.

Vanadate, a well known inhibitor of the Na^+, K^+-ATPase enzyme which exerts its effect on the intracellular side of the cell membrane also showed no effect on the ouabain sensitive ^{86}Rb uptake in VSMCs.[6] The inability of Cd^{2+} to inhibit the Na^+, K^+-ATPase pump activity in intact erythrocytes despite its high intracellular concentration is probably due to its binding to other intracellular protein, or its sequestration in subcellular organelles, or both. It has been shown that the main part of Cd^{2+} in blood cells is in red blood cells. In these cells Cd^{2+} is mainly bound to a protein with a molecular weight similar to metallothionein.[27]

Cadmium^{2+} also exerts various other effects at the cellular level such as an inhibition of choline uptake in human erythrocytes and rat brain synaptosomes[3 20]; an increase of the accumulation of Ca^{2+} into human red blood cells by increasing the passive Ca^{2+} influx[28]; age related changes of rat red blood cells such as density increment, shape change, decreased filterability, and shortened in vivo survival without affecting their ATP and glutathione concentra-

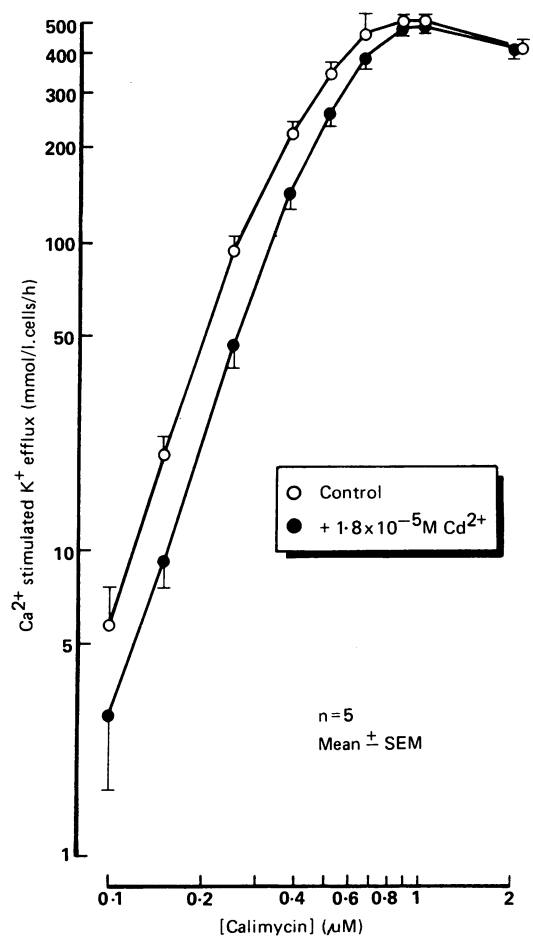

Figure 5 Effect of AH23187 (0·1 to 2 μM) on the erythrocyte Ca^{2+} stimulated K^+ efflux in the absence (○) or presence (●) of Cd ($1·8 \times 10^{-5}$ M).

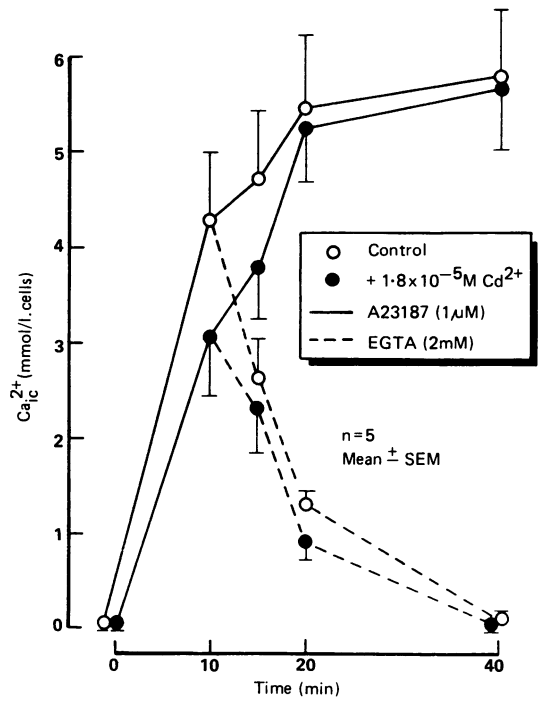

Figure 6 Effect of AH23187 (1 μM) on the Ca^{2+} uptake by intact red blood cells incubated in a Cs^+/Na^+ medium for 40 minutes without (○—○) and with (●—●) Cd ($1{\cdot}8\times10^{-5}$ M). In one set of experiments EGTA (2 mM) was added after incubation at 37°C for 10 minutes.

tions[29 30]; and an inhibition of the mitogenic response of human peripheral blood lymphocytes to the lectin concanavalin by increasing cytosolic free Ca^{2+} concentration.[31] The present study also showed a dose dependent inhibition of the erythrocyte Ca^{2+} dependent K^+ efflux by Cd^{2+} (fig 4) that can be attributed to a lower intracellular Ca^{2+} concentration obtained with the calcium ionophore calimycin in the presence of Cd^{2+}. Other transmembrane transport systems of Na^+ and K^+ such as Na^+, K^+ cotransport and Na^+, Li^+ countertransport activity, the anion carrier, and the number of active Na^+ pump units were not affected by Cd^{2+} when its concentration in the incubation medium did not exceed 100 μM. Higher Cd^{2+} concentrations, however, inhibit the bumetanide sensitive ^{86}Rb uptake or Na^+, K^+ cotransport system, the anion carrier, and the maximal ^3H-ouabain binding.

In conclusion, our in vitro data show that Cd^{2+} exerts an acute inhibitory effect on the intact human erythrocyte Ca^{2+} stimulated K^+ efflux and on the Na^+, K^+-ATPase enzyme in disrupted human red blood cells. By contrast, the Na^+, K^+-ATPase pump activity was not inhibited in intact blood cells by Cd^{2+} concentrations that generally inhibit the Na^+, K^+-ATPase enzyme in disrupted erythrocytes. The present study does not attempt to extrapolate any of the observed acute effects of Cd^{2+} on erythrocyte Na^+, K^+ transport systems in vitro to the possible acute or long term exposure of this heavy metal in man in vivo.

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1 Nechay BR, Saunders JP. Inhibitory characteristics of cadmium, lead, and mercury in human sodium and potassium dependent adenosine triphosphatase preparations. *Journal of Environmental Pathology and Toxicology* 1978;**2**:283–90.
2 Mustafa MG, Cross CE, Munn RJ, Hardic JA. Effect of divalent metal ions on alveolar macrophage membrane adenosine triphosphatase activity. *J Lab Clin Med* 1971;**77**:563–71.
3 Lai JCK, Guest JF, Leung TKC, Lim L, Davison AN. The effect of cadmium, manganese and aluminium on sodium-potassium activated and magnesium activated adenosine triphosphatase activity and choline uptake in rat brain symptosomes. *Biochem Pharmacol* 1980;**29**:141–6.
4 Takada M, Hayashi H. Effects of cadmium on the Na, K-ATPase of microsomes obtained from frog skin. *Jpn J Physiol* 1978;**28**:473–83.
5 Wald H, Gutman Y, Czaczkes W. Comparison of microsomal ATPase in the cortex, medulla, and papilla of the rat kidney. *Pflügers Arch* 1974;**352**:47–9.
6 Tokushige A, Higashimo H, Searle B, *et al.* Cadmium effect on the Na, K-ATPase system in cultured vascular smooth muscle cells. *Hypertension* 1974;**6**:20–6.
7 Lijnen P, Hespel P, Lommelen L, Laermans M, M'Buyamba-Kabangu JR, Amery A. Intracellular sodium, potassium and magnesium concentration, ouabain-sensitive ^{86}Rb-uptake and sodium-efflux and Na^+, K^+-cotransport activity in erythrocytes of normal male subjects studied at two occasions. *Methods Find Exp Clin Pharmacol* 1986;**8**:525–33.
8 Lijnen P, Tan W, Fagard R, Staessen J, Amery A. Changes in potassium content and membrane potassium channels in circulating cells from normal volunteers treated with cromakalim. *J Hypertens* 1989;**7**:403–7.
9 Cole CH. Erythrocyte membrane sodium transport in patients with treated and untreated essential hypertension. *Circulation* 1983;**68**:17–22.
10 De Luise M, Flier JS. Evidence for coordinate genetic control of Na, K-pump density in erythrocytes and leucocytes. *Metabolism* 1985;**34**:771–6.
11 Cumberbatch M, Morgan DB. Relations between sodium transport and sodium concentration in human erythrocytes in health and disease. *Clin Sci* 1981;**60**:555–64.
12 Garay RP, Hannaert PA, Nazaret C, Cragae EJ Jr. The significance of the relative effects of loop diuretics and anti-brain edema agents on the Na^+, K^+, Cl^--cotransport system and the $Cl^-/NaCO_3^-$ anion exchanger. *Naumyn Schmiedebergs Arch Pharmacol* 1986;**334**:202–9.
13 Nazaret C, Diez J, Hannaert PA, Christen MO, Wierzbicki N, Garay RP. Inhibition of the $Cl^-/NaCO_3^-$ anion exchanger by xipamide in human red blood cells. *Eur J Pharmacol* 1987; **144**:353–62.

14 Rosati C, Hannaert P, Dausse JP, Braquet P, Garay R. Stimulation of beta-adrenoceptors inhibits calcium-dependent potassium channels in mouse macrophages. *J Cell Physiol* 1986; **129**:310–4.
15 Canessa ML, Morgan K, Semplicini A. Genetic differences in lithium-sodium exchange and regulation of the sodium-hydrogen exchanger in essential hypertension. *J Cardiovasc Pharmacol* 1988;**12**:S92–8.
16 Canessa M, Adragna N, Solomon HS, Connolly TM, Tosteson DC. Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *New Engl J Med* 1980; **302**:772–6.
17 Swarts HGP, Bonting SL, De Pont JJ HHM, Schuurmans-Stekhoven FMAH, Thien TA, Van 't Laar A. Cation fluxes and Na^+, K^+-activated ATPase activity in erythrocytes of patients with essential hypertension. *Hypertension* 1981; **3**:641–9.
18 Woodman DD. Detection of blood in faeces. *Clin Chim Acta* 1970;**29**:249–52.
19 Nechay BR, Saunders JP. Inhibition of renal adenosine triphosphatase by cadmium. *J Pharmacol Exp Ther* 1977;**200**:623–9.
20 King RG, Sharp JA, Boura ALA. The effects of Al^{3+}, Cd^{2+} and Mn^{2+} on human erythrocyte choline transport. *Biochem Pharmacol* 1983;**23**:3611–7.
21 Rifkin JR. In vitro inhibition of Na^+, K^+ and Mg^{2+}-ATPases by mono-, di- and trivalent cations. *Proc Soc Exp Biol Med* 1965;**120**:802–4.
22 Bader H, Wilkes AB, Jean DH. The effect of hydroxylamine mercaptans, divalent metals and chelators on Na^+, K^+-ATPase. *Biochim Biophys Acta* 1970;**198**:583–93.
23 Hexum TD. Studies on the reaction catalyzed by transport (Na, K) adenosine triphosphatase. Effects of divalent metals. *Biochem Pharmacol* 1974;**23**:3441–7.
24 Jacobson KB, Turner JE. The interaction of cadmium and certain other metal ions with protein and nucleic acids. *Toxicology* 1980;**16**:1–37.
26 Skou JC. Study on the Na^+ + K^+ activated ATP hydrolysing enzyme system. The role of SH groups. *Biochem Biophys Res Commun* 1963;**10**:79–84.
26 Glynn IM. Transport adenosine triphosphatase in electric organ. The relation between ion transport and oxidative phosphorylation. *J Physiol (Lond)* 1963;**169**:452–65.
27 Elinder GG. Normal values for cadmium in human tissues, blood and urine in different countries. In: Friberg L, Elinder CG, Kjellström T, Nordberg GF, eds. *Cadmium and health: a toxicological and epidemiological appraisal.* Boca Raton, Florida: 1985:82–98.
28 Plishker GA. Effects of cadmium and zinc on calcium uptake in human red blood cells. *Am J Physiol* 1984;**247**:C143–9.
29 Kunimoto M, Miura T, Kubota K. An apparent acceleration of age-related changes of rat blood cells by cadmium. *Toxicol Appl Pharmacol* 1985;77:451–7.
30 Kunimoto M, Miyasaka K, Miura T. Changes in membrane properties of rat blood cells induced by cadmium accumulating in the membrane fraction. *J Biochem* 1986;**99**:397–406.
31 Scott IG, Wolff HJ, Åkerman KEO, Andersson LC. Effects of Cd^{2+} upon Ca^{2+} fluxes and proliferation in concanavalin A-stimulated lymphocytes. *Exp Cell Res* 1985;**156**:191–7.

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Lead to unusual places

I suppose one could write a book about unusual occurrences of lead poisoning, and they keep cropping up from time to time. Every few months there is a report of someone being poisoned from contaminated home made wine or other foodstuffs and recently I remember reading about a family in the United States who had got lead poisoning after their father had enthusiastically rubbed down all the paint in their old house with dry sandpaper. This case first came to light when their vet diagnosed lead poisoning in their dog; the human doctors hadn't considered it when dealing with the other members of the household. Those who know something about the history of lead poisoning are rarely surprised by these revelations but I have come across one lead hazard of which I was not previously aware.

My first indication of it came when I was talking to a young man who was working in a joinery firm in the east end of London. His job was to feed wood through the circular saw and he came to see me because he had cut a thumb off and there was a question of the allocation of blame and settlement of damages. I asked him about his work and whether anyone else had suffered any injuries. It was all right, he said, but you had to watch out for bits flying off the saw.

"Why?"

"Because of the bullets."

"What!"

And then he explained that the wood they were using had come from France, from areas where there had been heavy fighting during the first world war and the trunks had bullets in them which had later disappeared into the depth of the tree as it grew. Then, when they came to cut the trees into planks, the saw would break if it came across a bullet, sending a piece of metal at colossal speed across the shop.

This seemed to be such an apocryphal story that I kept quiet about it for several years. Talking to a builder who was doing some work on my house, however, the conversation came round to occupational hazards, as it does, and he—without any prompting—told me the same story. He too had worked as a young man in a woodyard in which there had been bullets in the trees. This confirmed what I had heard before and I was now prepared to believe it. "And there was the musket," he went on. "What?" And he told me that one tree had been opened up and there in the middle, was a musket from the Napoleonic wars. At this point his credibility vanished; what next, a skeleton?

Can anyone verify this? Are there any other stories doing the rounds? Please let me know.

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