Monensin Mediates a Rapid and Selective Transport of Pb$^{2+}$

POSSIBLE APPLICATION OF MONENSN FOR THE TREATMENT OF Pb$^{2+}$ INTOXICATION

The carboxylic acid ionophore monensin, known as an electroneutral Na$^+$ ionophore, an anticoecidial agent, and a growth-promoting feed additive in agriculture, is shown to be highly efficient as an ionophore for Pb$^{2+}$ and to be highly selective for Pb$^{2+}$ compared with other divalent cations. Monensin transports Pb$^{2+}$ by an electroneutral mechanism in which the complex PbMonOH is the transporting species. Electrogenic transport via the species PbMon$^+$ may also be possible. Monensin catalyzed Pb$^{2+}$ transport is little affected by Ca$^{2+}$, Mg$^{2+}$, or K$^+$ concentrations that are encountered in living systems. Na$^+$ is inhibitory, but its effectiveness at 100 mM does not exceed 50%. The poor activity of monensin as an ionophore for divalent cations other than Pb$^{2+}$ is consistent with the pattern of complex formation constants observed in the mixed solvent 80% methanol/water. This pattern also explains why Ca$^{2+}$, Mg$^{2+}$, and K$^+$ are ineffective as inhibitors of Pb$^{2+}$ transport, but it does not fully explain the actions of Na$^+$, where kinetic features of the transport mechanism may also be important. When given to rats at 100 ppm in feed together with Pb$^{2+}$ at 100 ppm in drinking water, monensin reduces Pb accumulation in several organs and tissues. It also accelerates the excretion of Pb that was accumulated previously and produces this effect without depleting the organs of zinc or copper. Monensin, used alone or in combination with other agents, may be useful for the treatment of Pb intoxication.

Ionophores are lipophilic chelating agents that transport cations across phospholipid bilayer membranes, such as the plasma and subcellular membranes of cells. The naturally occurring compounds (about 100) are antibiotics produced primarily by soil bacteria of the Streptomyces genus (1). They are distinguished from pore-forming antibiotics such as gramicidin by the involvement of a discrete complex in the transport mechanism. Thus, true ionophores can be highly selective for particular cations (2, 3). The known compounds are generally divided into two groups: the so-called electrogenic and the electroneutral ionophores (4, 5). Electrogenic ionophores are typified by the well known compound valinomycin (6). They are neutral molecules that form cation complexes that carry a net positive charge, such that transbilayer charge movements accompany transport catalyzed by this class. Accordingly, the rate of transport is influenced by membrane electrical potential, which also determines the transmembrane distribution of the transported cation at equilibrium. Compounds such as monensin, nigericin, and A23187 (Fig. 1) typify electroneutral ionophores, also called carboxylic acid or polyether ionophores. The anionic forms of these compounds complex the cation, which is exchanged for H$^+$, or another cation, without net charge movement. Transport catalyzed by this class is therefore influenced by transmembrane pH gradients, as is the equilibrium distribution of cations.

Among the carboxylic acid ionophores, it has been common to consider individual compounds as able to complex/transport a small group of cations having the same charge, with transport occurring through a single species by a mechanism that is purely electrogenic or electroneutral. Thus, monensin and nigericin were known as ionophores for Na$^+$ and K$^+$, respectively, with transport occurring via a 1:1 complex and electroneutral mechanism; A23187 was known as a Ca$^{2+}$ ionophore transporting via a 1:2 complex and an electroneutral mechanism, and so forth. In work conducted over a period of time, we showed that the model depicted in Fig. 2 is a more accurate representation of the factors that establish the transport properties of a carboxylic acid ionophore (7–16). The equilibrium shown at the top of Fig. 2 represents formation of 1:1 complexes and emphasizes that carboxylic acid ionophores react with a broad range of cations ($n = 1$–3), not just those with a particular charge as often assumed. The 1:1 complexes then react to form higher order species by competing equilibria I–III, which involve free ionophore ($A^-$), hydroxide ion ($OH^-$), and other anions ($X^-$), respectively. Complexes of differing stoichiometry therefore arise, which include mixed species containing $OH^-$ and $X^-$. Within this scheme, the mode and overall rate of transport will reflect the distribution of ionophore between these various complexes and their respective transmembrane diffusion constants. As a corollary of this interpretation, for a given ionophore and set of conditions, transport selectivity will also depend on how these factors vary with properties of the cation. The latter include size, charge, coordination and hydration number, preferred donor atom geometry, Lewis acidity, and ease of hydrolysis.

In considering the above model, the characteristics of the donor atoms in typical ionophores, and the metal ion complexation properties of analogous synthetic polyethers (17), we realized that some of the antibiotic compounds might be efficient ionophores for Pb$^{2+}$ and for other cations with biological toxicity.
Subsequently, we showed that ionomycin transports Pb\(^{2+}\) rapidly and with selectivity over Ca\(^{2+}\) near 10\(^5\) when both cations are present simultaneously. Ionomycin was also found to affect an efficient transport of Pb\(^{2+}\) ions when the cells had been previously loaded. We indicated that ionomycin should be considered primarily as an ionophore for Pb\(^{2+}\), rather than Ca\(^{2+}\), and suggested that its Pb\(^{2+}\)-transporting activity might be adapted to improve existing treatments for Pb\(^{2+}\) intoxication (15).

In the present report, we extend the investigation of ionophore-mediated Pb\(^{2+}\) transport by demonstrating that monensin is also effective as a Pb\(^{2+}\) ionophore and is more selective in that regard than is ionomycin. We also show that monensin promotes the excretion of Pb\(^{2+}\) from rats when the cation has been previously provided in drinking water and the ionophore is administered in feed. Aspects of these data have been presented in abstract form (18).

**EXPERIMENTAL PROCEDURES**

**Reagents and Solvents**—High purity nitric acid (trace metal; Fisher) and perchloric acid (double-distilled; GFS Chemicals) were obtained from commercial sources. Synthetic 1-palmitoyl-2-oleoyl-sn-glycerophosphatidylcholine (POPC)\(^3\) was obtained from Avanti Polar Lipids, Inc. Purity was confirmed by thin layer chromatography before use. For the transport studies, monensin and ionomycin were obtained from Calbiochem and used without further purification. Ionomycin stock solutions were prepared in ethanol and standardized spectrophotometrically using an extinction coefficient of 13,560 at 278 nm. In the case of monensin, standardization was gravimetric or by titration with Me\(_4\)NOH. Quin-2 (K\(^{+}\) salt) from Sigma was purified by passage over Chelex 100 resin (100–200-mesh) in the Cs\(^{+}\) form as described previously (19), or in the Na\(^{+}\) form when Na\(^{+}\)-loaded vesicles were employed. The nitrate and chloride salts of divalent cations were the ultrapure grade from Alfa Products. Stock solutions were standardized by titration with a primary standard EDTA solution (20) or by atomic absorption spectroscopy using certified solutions (Fisher).

For solution chemical studies, a mixed solvent of 80% (w/w) methanol in water was prepared gravimetrically, using distilled deionized water and reagent grade methanol (Fisher) that had been freshly distilled. The Et\(_4\)NClO\(_4\) that was used to maintain ionic strength in this solvent was prepared by reaction of Et\(_4\)NOH (Aldrich) with 70% perchloric acid (distilled; GSH Chemicals). The salt obtained was recrystallized four times from water. Solvent containing Et\(_4\)NClO\(_4\) and H\(^{+}\) buffering compounds was further deionized by passage over Chelex 100. For this purpose, the resin was in the Et\(_4\)N\(^{+}\) form, which was prepared as previously described (8).

**Preparation of Phospholipid Vesicles**—The preparation of freeze-thaw-extruded POPC vesicles loaded with Quin-2 has also been described previously (21, 22). Briefly, 300 mg of POPC in chloroform was dried by rotation under a nitrogen stream to produce a film on the wall of a 25 × 150-mm culture tube. Residual solvent was removed under high vacuum (4 h), and the film was subsequently hydrated in 6 ml of a solution containing 6.6 mM purified Quin-2 and 10.0 mM Hepes buffer adjusted to pH 7.00 with Chelex-treated CsOH or NaOH (19), depending on the internal composition required. The mixture was vortexed, and the resulting multilamellar vesicles were frozen in a dry ice-acetone bath, thawed in lukewarm water, and vortexed again. The freeze-thaw and vortexing procedures were repeated two additional times, after which the vesicles were extruded three times through two stacked 100-nm polycarbonate membrane filters. This step was followed by six additional freeze-thaw cycles coupled with additional extrusions. The resulting preparations were applied to Sephadex G-50 minicolumns (23) to remove extravesicular Quin-2. These columns were eluted by low speed centrifugation and had previously been equilibrated with a solution containing 10 mM Hepes buffer, pH 7.00. A single pass over such columns effectively removes the external Quin-2 (19, 21, 22).

The nominal concentration of POPC in the final preparations was determined by measurement of lipid phosphorus (24) and was near 80 mM. The average diameter of these vesicles is 71 nm as determined by freeze-fracture electron microscopy (21), and they contain entrapped solutes at the following concentrations: Quin-2, 10.5 ± 0.8 mM; Hepes, 34 ± 8 mM (pH 7.4); and Cs\(^{+}\)/Na\(^{+}\), 60 ± 5 mM. Specific values for

1 The abbreviations used are: POPC, 1-palmitoyl-2-oleoyl-sn-glycero-phosphatidylcholine; CCCP, carbonyl cyanide m-chlorophenylhydrazone; Ches, 2-(cyclohexylamino)ethanesulfonic acid; Mes, 2-(N-morpholino)ethanesulfonic acid.
Quin-2 and Cs⁺/Na⁺ were determined for each preparation by the methods described previously (19, 25). Briefly, entrapped Quin-2 is determined by spectrophotometric titration with standard CaCl₂ following dispersion of the vesicles in deoxycholate. The entrapped monovalent cation is determined by atomic absorption spectrophotometry, following replacement of the excess Na⁺ salt by CsCl. CaCl₂ was adjusted with CsOH that had been passed over Chelex 100 columns to aid equilibration of transmembrane pH.

When determining S, the concentration of the Pb²⁺ or Ca²⁺ was 20 μM, and all other conditions were held constant. All transport data were obtained at 25.0 °C.

**Potentiometric Titrations and the Determination of pH in Aqueous Methanol**—The protonation constants and complex formation constants of monensin were measured by potentiometric methods in the mixed solvent 80% (v/v) methanol/water. For these studies, Na⁺-monensin was purified by column chromatography on silica gel, using ethyl acetate as the solvent. The monensin was dissolved in methanol, and the pH was adjusted with 0.1 M NaOH. Stock solutions of 5.0 mM monensin in methanol were standardized by titration using EDTA (20). NaCl (99.99%; Aldrich) and KCl (99.99%; Aesar) were dried at 110 °C, and stock solutions of these salts were prepared gravimetrically.

Test solutions for potentiometric titration typically contained 0.5–1.0 mM monensin acetic acid (HL) and, where appropriate, the metal ion (M) at concentration ratios ([HL]/[M]) in the range of 1.0–3.0. In the case of MgCl₂ and KCl, [HL]/[M] ratios of 0.2–0.25 were also used. Ionic strength was maintained at 0.050 using Et₄NClO₄, except in the case of K⁺, where Et₄NClO₄ (99%); Fluka) was used. The titrations were carried out using a digital burette, (Metrohm, model 665) and pH meter ( Fisher, model 201). Single-component reactions were measured using a barium alkali salt as modifier. All measurements were made using double junction combination electrodes (Sensorex S1021CD, Orion Ross 8175BN, Thomas 4080-B49), where the external filling solution was replaced with 0.1 M Et₄NClO₄ or 0.1 M Me₄NCl in 20% methanol/water.

The pH meter-electrode system was calibrated in aqueous solution using standard buffers (Gram-Pac; Fisher); then the electrodes were equilibrated in 80% methanol/water for at least 2 h prior to measurement. The operational pH* scales developed by de Ligny et al. (30, 31) and Gelsema et al. (32, 33) were utilized to determine the value of pH*. The term pH* is defined as −log aH⁺, where aH⁺ is the activity of H⁺ in the mixed solvent. Accordingly, the term pH* when used in reference to a specific methanol/water mixture has the same meaning as the term pH when used in reference to an aqueous solution (see Ref. 34 and references therein).

All titrations were carried out at 25.0 °C using a thermostatted cell and 20 mM Me₄NOH as the titrant. A nitrogen or argon atmosphere was maintained to minimize contamination by CO₂. The Me₄NOH was standardized using KH₂PO₄ and checked for carbonate content (35). Typical titrations consisted of 50–100 pairs of pH* versus mL Me₄NOH added. The titration data were analyzed using the computer programs PKAS for the protonation constants (36) and BEST for the metal ion complexation constants (37).**

**Treatment of Experimental Animals**—Male rats were utilized when investigating the effects of monensin on the pathophysiology of Pb²⁺. They were housed in AALAC-approved animal facilities at the College of Medicine, Ohio State University. A 12-h light-dark cycle, single housing in plastic cages, or in metabolic cages, and conditions of constant temperature and humidity were employed. One week was maintained to minimize contamination by CO₂. The Me₄NOH was standardized using KH₂PO₄ and checked for carbonate content (35). Typical titrations consisted of 50–100 pairs of pH* versus mL Me₄NOH added. The titration data were analyzed using the computer programs PKAS for the protonation constants (36) and BEST for the metal ion complexation constants (37).**

**Determination of Pb in Biological Samples**—Blood samples were taken periodically from the tail artery, and blood Pb levels were determined by electrothermal atomic absorption spectroscopy against certified standards (39). For the determination of Pb in urine and feces, total outputs were collected over 2-day periods from each rat individually. The urine samples were neutralized by the careful addition of nitric acid while the samples were in the fume hood, and the pH was adjusted to about pH 2. Feces were then extracted with methyl isobutyl ketone in the presence of excess ammonium pyrrolidinedithiocarbamate, which quantitatively extracts Pb²⁺ into the organic layer and concentrates it compared with the original concentration in urine (40). The Pb content was thereafter determined by flame atomic absorption spectroscopy. For the determination of Pb in feces, weighed samples comprising ~1.0 g were dispersed in 10 ml of
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RESULTS

Monensin-mediated Pb$^{2+}$ Transport—Fig. 3 compares the efficiency of ionomycin and monensin as ionophores for Pb$^{2+}$ and contrasts their selectivity for Pb$^{2+}$ compared with other divalent cations. Under the conditions employed, the two compounds are similarly effective from the perspective of rate, whereas monensin is much more selective. Selectivity for Pb$^{2+}$ over Ca$^{2+}$, for example, as defined by Equation 2, is $\sim 100$ for ionomycin and $\sim 3400$ for monensin.

The stoichiometry of the complex between ionomycin and Pb$^{2+}$ that is responsible for transport is 1:1, cation/ionophore, based upon plots of log rate versus log ionomycin or log Pb$^{2+}$ concentration, which both display slopes of 1.0 (15). By the same criteria, monensin-mediated Pb$^{2+}$ transport also occurs through formation of a 1:1 complex (Fig. 4), although the plot of log rate versus log Pb$^{2+}$ concentration progressively deviates from a slope of 1 as the free Pb$^{2+}$ concentration increases above 1 $\mu$M. This negative deviation suggests that the steady state fraction of monensin that is located at the external membrane interface approaches saturation with Pb$^{2+}$ at concentrations above that value.

Ionomycin is dibasic, because both the carboxylic acid function and the enolized $\beta$ diketone moiety can be ionized (Fig. 1) (43). Accordingly, ionomycin can form uncharged complexes with divalent cations, presumably including Pb$^{2+}$, and can exchange these for 2H$^+$ in an electroneutral manner (19, 25). In contrast, monensin is monobasic (Fig. 1) (44) and would form a 1:1 complex with Pb$^{2+}$ having a net charge of +1. Thus, the 1:1 complex between Pb$^{2+}$ and monensin might result in electrogenic Pb$^{2+}$ transport, or it might associate with an anion as depicted in Fig. 2, to produce transport through a charge neutral mechanism. To examine the mode of Pb$^{2+}$ transport, we initially determined complex stability constants of potential transporting species. Potentiometric titration methods were employed using 80% methanol/water as the solvent. This mixed solvent provides an effective polarity similar to that experienced by ionophores at a POPC membrane interface (7, 12, 45, 46). Analogous complexation equilibria for other cations were also examined to provide insight into the basis of the high selectivity that is seen in Fig. 3B.

Fig. 5A shows examples of the primary data obtained, whereas Fig. 5B reports the complex stability constants. It is seen that the complex PbMon$^+$ is relatively stable, displaying a log $K$ value of 7.25. The uncharged complex PbMonOH that is formed upon reaction of PbMon$^+$ with OH$^-$ is nearly 4 orders of magnitude more stable than the 1:2 complex formed from PbMon$^+$ and a second molecule of the ionized ionophore. When these stability constants and the protonation constant of monensin are used to generate a species distribution diagram (29) (Fig. 5C), it is seen that significant levels of both PbMon$^+$ and PbMonOH are expected at the membrane interface during Pb$^{2+}$ transport, within the pH range of 6.5–7.5. In contrast, the species PbMon$_{2}$OH is negligible ($<0.003\%$ of total monensin) throughout the broad range of pH considered (not shown). Thus, the complex stability data indicate that monensin might transport Pb$^{2+}$ by a mixed mode (electrogenic and electroneutral transport occurring simultaneously) and that the fraction transported by the neutral process would probably occur via the species PbMonOH.

To further examine these possibilities, the effect of external pH on the rate of Pb$^{2+}$ transport was examined, using Na$^+$-containing vesicles with valinomycin and CCCP excluded. These conditions limit transport to the fraction occurring by neutral mechanisms, because no provision is made to collapse

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$^{2}$ When both cations are present simultaneously, the selectivity of ionomycin for Pb$^{2+}$ compared with Ca$^{2+}$ is similar to the value for monensin derived from Fig. 3B (15).
the membrane potential arising from electrogenic transport, which is very effective at limiting the process (e.g. Refs. 14 and 16). In addition, the use of Na\(^+\) rather than Cs\(^+\)-containing vesicles allows the ionophore to exchange Pb\(^{2+}\) for Na\(^+\), ensuring that the rate of Pb\(^{2+}\) transport does not become limited by the protonation of monensin at the internal membrane interface. As seen in Fig. 6A, monensin remains active as an ionophore for Pb\(^{2+}\) under these conditions, demonstrating that the electroneutral mode is indeed active.

Regarding the species PbMonOH, the data in Fig. 6B show that increasing external pH enhances the rate of transport, consistent with a model involving dissociation of a single proton from hydrated PbMon\(^+\) to form PbMonOH. This finding and a comparison of the transport data with the pH dependence for PbMonOH formation shown in Fig. 5C indicate that PbMonOH is indeed the major transporting species. In Fig. 6, the half-maximal rate was seen at pH 7.8 (pOH 6.2) or at pH OH 6.9 if it is considered that that species is the one which is actually transported.

Competitive Relationships between Pb\(^{2+}\) and Other Cations—Given the potential use of ionophores to manipulate Pb\(^{2+}\) in living systems, we considered the possibility that the relatively high concentrations of other cations found in blood and intracellular compartments might substantially prevent monensin from transporting Pb\(^{2+}\) in vivo. Specifically, the rates of Pb\(^{2+}\) transport were compared when the free Pb\(^{2+}\) concentration was buffered at 1.0 \(\mu\)M alone and when 1.0 mM free Ca\(^{2+}\) or Mg\(^{2+}\) was also present. Only negligible differences were seen (Fig. 7, A and B), as might be expected based upon the complex stability constants shown in Fig. 5B. Potential interference by K\(^+\) and Na\(^+\) was also examined. The actions of K\(^+\) were again negligible when it was present at 5.0 or 100 mM, approximately the levels of this cation in blood and cytoplasm respectively (data not shown). The same concentrations of Na\(^+\) did have modest inhibitory effects (Fig. 7C); however, these were smaller than expected based on the complex stability constant values listed in Fig. 5B. In other words, the ratio \(K_{Pb}/K_{Na}\) is \(-300\), whereas at a ratio of free Na\(^+\)/Pb\(^{2+}\) of \(10^5\) (i.e. when Na\(^+\) was present at 100 mM and Pb\(^{2+}\) at 1.0 \(\mu\)M), the rate of Pb\(^{2+}\) transport is reduced by only a factor of \(<2\).

To further examine the effectiveness of Na\(^+\) as an inhibitor of monensin-mediated Pb\(^{2+}\) transport, the Na\(^+\) concentration was varied from 0 to 100 mM while another solute was varied in the opposite direction, so as to maintain the external ionic strength and/or the osmotic pressure at constant values. As seen in Fig. 8, Na\(^+\) is more effective when used to replace Cs\(^+\), compared with K\(^+\) or tetraethylammonium cation, and it is ineffective when used to replace mannitol. These data presumably reflect modest differences in the stability of complexes between monensin and monovalent cations (47–49) together with effects of ionic strength on complex stability. However, of greater importance, they further indicate that concentrations of Na\(^+\) found in living systems have little effect on the efficiency of monensin as a Pb\(^{2+}\) ionophore.

Monensin Promotes the Excretion of Pb\(^{2+}\) in Rats—Given that the above data are consistent with the notion that monensin might alter the dynamics of Pb\(^{2+}\) in whole organisms, we determined the effect of monensin on the accumulation and disposition of Pb in rats. In one experiment, the ionophore and Pb\(^{2+}\) were administered simultaneously, at 100

**Fig. 4.** Pb\(^{2+}\) transport dependence on the concentrations of Pb\(^{2+}\) and monensin. Data were obtained as described in the legend to Fig. 3 except that the concentrations of Pb\(^{2+}\) and monensin were varied. In addition, the external free Pb\(^{2+}\) concentration was established by a 15 mM citrate-based buffer system, as described under “Experimental Procedures.” A, the monensin concentration was 0.10 \(\mu\)M, and the concentration of free Pb\(^{2+}\) was varied from 10 nM (bottommost curve) to 5.63 \(\mu\)M (topmost curve). B, the free Pb\(^{2+}\) concentration was 1.0 \(\mu\)M, and the concentration of monensin was varied from 1.17 nM (bottommost curve) to 2.40 \(\mu\)M (topmost curve). C, the data from A and B are shown as log initial rate versus log Pb\(^{2+}\) (O) or log monensin (•) concentration. Initial rates were obtained from the individual progress curves as described under “Experimental Procedures.”
ppm in feed and 100 ppm in drinking water, respectively, or Pb\(^{2+}\) was administered without the ionophore. Blood samples were taken at weekly intervals over a 28-day period, and thereafter the rats were sacrificed to allow the determination of Pb in organs and tissues. Throughout the entire experimental period, the average concentration of Pb in blood was lower by \(-25\%\) in the rats that had received monensin, and this difference was significant at \(p = 0.05\) (Table I and data not shown). Monensin also reduced the accumulation of Pb in several organs, with a particularly large effect seen in heart, where it was reduced by 66% (Table I). The reduced values seen in brain, muscle, and bone were also significant.

In a second experiment, the rats were loaded with Pb\(^{2+}\) over a 21-day period and in the absence of monensin. Thereafter, they were divided into groups and given drinking water that did not contain Pb\(^{2+}\). One group was given monensin at 100 ppm in feed, whereas another was given the same feed without monensin. After an additional 21 days, the rats were sacrificed, and lead in organs and tissues was determined. This time there

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**Fig. 5.** Equilibrium behavior of monensin and selected cations. A, potentiometric titrations of 1.0 mM monensin in an 80% methanol/water mixed solvent were conducted as described under “Experimental Procedures.” The temperature was 25°C, and the ionic strength was 0.1 M, maintained by the presence of tetraethylammonium perchlorate. I, monensin alone; II, monensin in the presence of 0.5 mM Pb\(^{2+}\); III, monensin in the presence of 1.0 mM Pb\(^{2+}\). B, equilibrium constants of interest, determined from data analogous to those shown in A. The symbol \(L^-\) refers to the monensin carboxylate anion. C, the species distribution diagram for a solution containing a total of 0.10 M monensin and with the Pb\(^{2+}\) concentration fixed at 0.10 M. Individual values were calculated using the program COMICS (29), the equilibrium constants of interest from B, and a \(pK_a\) of 7.96 for the hydrolysis of Pb\(^{2+}\) in 80% methanol/water (77). In C, Mon\(^{--}\) represents monensin in the carboxylate anion form.

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**Fig. 6.** Effect of external pH on ionophore-mediated Pb\(^{2+}\) transport. Vesicles were prepared as described under “Experimental Procedures” except that Na\(^+\) rather than Cs\(^+\) was employed as the counter ion to Quin-2. The external medium contained 10 mM each of Mes, Hapes, and Ches, to provide for H\(^+\) buffering over a broad range of pH, and 5 mM citrate was present to buffer free Pb\(^{2+}\). K\(^+\) was used as the external counter ion for citrate and the H\(^+\) buffers. The external pH was set at a value of interest, and the concentration of Pb\((NO_3)_4\) required to produce a free Pb\(^{2+}\) concentration of 0.10 mM was added to the cuvette. Pb\(^{2+}\) transport was initiated by the addition of 0.1 mM monensin. A, individual progress curves covering the pH region of 6.5–9.0. The earliest portions are omitted, because the initial equilibration of H\(^+\) and monovalent cation gradients perturbs the UV-visible spectrum of Quin-2. Accordingly, rates pertaining at 140 s were determined rather than the initial rates. B, rates obtained from A at the pH indicated. Values are the mean of duplicate determinations. They were fit to the Henderson-Hasselbalch equation to obtain the solid line.
was no difference in blood lead, either at the time of sacrifice (Table II) or at earlier sampling times (data not shown). However, the rats given monensin had lower levels of lead in brain, kidney, liver, and bone. The greatest effect was seen in kidney (a 55% reduction), with the effect on liver being of similar magnitude. Consistent with the transport data shown in Fig. 3 and the complex stability constants shown in Fig. 5B, the reduced levels of Pb in organs were obtained without reducing the levels of zinc or copper (Table III). Thus, the actions of monensin on lead are not accompanied by perturbation of these trace elements having biological roles.

During this second experiment, the rats were housed in metabolic cages so that urine and feces could be collected to determine the fate of Pb released from the organs. Amounts excreted in urine were low and were unaffected by the presence or absence of monensin (Fig. 9). Determining excretion via the feces is more problematic, because the gastrointestinal tract in rats rejects most Pb that is ingested, resulting in high levels of Pb in feces during the loading period (Refs. 50 and 51 and data not shown). When Pb\(^{2+}\) is withdrawn, feces Pb remains high for several days while the contents of the gastrointestinal tract turn over. These large values of excreted Pb obscure smaller differences between groups, such as differences that might be produced by monensin. Nevertheless, at later times, a statistically significant effect of monensin on Pb in feces was observed. In other words, during the second and third week of monensin administration, the treated rats lost lead at a rate of \(120\) nmol/day, whereas without monensin the rate was about half that value. As further considered below, these data suggest that the lead mobilized from organs by monensin is ultimately excreted via the feces.

**DISCUSSION**

Monensin was among the first group of carboxylic acid ionophores to be discovered, a group that also includes dianemycin, nigericin, compound X-206, and lasalocid A (reviewed in Ref. 5). Soon after these compounds were reported to be potent anticoccidial agents (52) and to derive this activity through a direct interaction with monovalent cations, leading to altered cation transport (53). Among the monovalent alkali metal cations, monensin was subsequently shown to be most effective as an ionophore for Na\(^{+}\), and it has long been utilized as a research tool in that regard (reviewed in Refs. 2 and 54). It has also been used as a feed additive in agriculture, because of the anticoccidial activity and because it promotes the growth of several animal species that are used in that industry (55, 56).
Two groups of six rats were given lead at 100 ppm in their drinking water, as further described under “Experimental Procedures.” One group also received monensin at 100 ppm in feed, whereas the other group did not. After 28 days, the rats were sacrificed, and lead was determined in the indicated organs and tissues, as further described under “Experimental Procedures.” “Muscle” and “bone” refer to the quadriceps muscle and the femur, respectively. All values are means ± one S.D. value and are in units of nmol/g, wet weight, except for blood, which is in µmol. Control values (no lead, no monensin) are from the literature (69). For tissues marked with an asterisk, the lead plus monensin value was lower than the lead value at p < 0.05. For those marked *, the threshold was p < 0.10. For liver and kidney, the differences did not reach either threshold.

| Tissue   | Control | Lead | Lead plus monensin |
|----------|---------|------|--------------------|
| Blood*   | 0.10    | 1.17 ± 0.32 | 0.88 ± 0.24 |
| Heart*   | 0.005   | 0.33 ± 0.11 | 0.11 ± 0.04 |
| Brain*   | 0.050   | 0.71 ± 0.35 | 0.50 ± 0.13 |
| Liver    | 0.20    | 3.82 ± 2.04 | 2.88 ± 0.65 |
| Kidney   | 0.20    | 23.3 ± 8.73 | 21.0 ± 5.35 |
| Muscle*  | Nil     | 0.20 ± 0.07 | 0.13 ± 0.05 |
| Bone*    | Nil     | 264 ± 82   | 173 ± 29   |

Two groups of eight rats were given Pb²⁺ at 100 ppm in drinking water without the administration of monensin. After 21 days, lead was withdrawn, and the administration of monensin at 100 ppm in feed began in one of the groups, whereas normal feed was given to the other group. After an additional 21 days, the rats were sacrificed, and lead was determined in the indicated organs and tissues, as further described under “Experimental Procedures.” “Muscle” and “bone” refer to the quadriceps muscle and the femur, respectively. All values are means ± one S.D. value and are in units of nmol/g, wet weight, except for blood, which is in µmol. Control values (no lead, no monensin) are from the literature (69). As in Table II, for tissues marked with an asterisk, the plus monensin value was lower than the no monensin value at p < 0.05. For those marked *, the threshold was p < 0.10. For heart and muscle, the differences did not reach either threshold.

| Tissue   | Normal values | No monensin | Plus monensin |
|----------|---------------|-------------|---------------|
| Blood    | 0.10          | 0.82 ± 0.23 | 0.83 ± 0.27 |
| Heart    | 0.005         | 0.13 ± 0.09 | 0.09 ± 0.07 |
| Brain    | 0.05          | 0.80 ± 0.44 | 0.65 ± 0.20 |
| Liver*a  | 0.20          | 1.19 ± 0.48 | 0.608 ± 0.46 |
| Kidney   | 0.20          | 16.4 ± 6.4  | 7.32 ± 3.3 |
| Muscle   | Nil           | 0.033 ± 0.03 | 0.014 ± 0.01 |
| Bone*    | Nil           | 310 ± 109   | 189 ± 78    |
eight rats were given 100 ppm Pb2+ in drinking water, for 21 days, as further described under "Experimental Procedures." The rats were housed in metabolic cages, allowing total urine output by each rat to be collected over 2-day intervals. Lead was determined by atomic absorption spectroscopy and expressed as nmol of lead excreted per rat per day. Up to and including day 21, the values plotted are means of those obtained for all 16 rats. On day 22, Pb2+ was withdrawn, and one group was shifted to a diet containing 100 ppm monensin, while the second was maintained without monensin. 

significnat concentrations of Na+ are also present.

The current data obtained in model systems suggest that, like ionomycin (15), monensin might be useful in the treatment of lead intoxication; however, there are no studies that have tested that possibility in animals. Accordingly, we determined whether monensin alters the accumulation or distribution of lead when the two agents are given simultaneously and whether monensin affects the disposition of lead that was ac-

culated previously. The level of Pb2+ in the same as that usually given to chickens as an agricultural practice (56) and is below the toxic threshold for monensin in this respect (76). Thus, monensin may indeed be useful for promoting the elimination of lead from animals.

At this early stage, it is appropriate to consider the possibility that monensin acts differently than the traditional compounds as regards the mechanism by which lead excretion occurs. The traditional compounds are hydrophilic cation chelators that circulate in blood and form Pb2+ complexes having stability constants of $-10^{15}$ and higher. They are not

### Table III

| Tissue  | Zinc | Copper |
|---------|------|--------|
|         | No monensin | Plus monensin | No monensin | Plus monensin |
| Heart   | 249 ± 14 | 238 ± 12 | 61.9 ± 4.7 | 74.1 ± 6.9 |
| Brain   | 230 ± 4.0 | 226 ± 13 | 26.8 ± 2.3 | 35.3 ± 2.5 |
| Liver   | 428 ± 25 | 395 ± 48 | 56.7 ± 5.1 | 57.6 ± 6.4 |
| Kidney  | 330 ± 27 | 319 ± 16 | 99.0 ± 18 | 97.0 ± 17 |
| Muscle  | 166 ± 33 | 150 ± 34 | 11.1 ± 1.7 | 10.7 ± 1.5 |
| Bone    | 2136 ± 112 | 1975 ± 66 | 44.6 ± 2.3 | 44.9 ± 3.2 |

**FIG. 10. Potential mechanisms of monensin catalyzed Pb2+ transport.** The curved lines together represent a phospholipid bilayer, whereas the heavy arrows indicate transmembrane diffusion of a lead-monensin complex. The various species illustrated are assumed to be present near the membrane-bulk aqueous phase interface.

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3 Consistent with the reports cited, we did not find a significant effect of monensin at 100 ppm on growth (weight gain) or on the consumption of feed and water.
membrane-permeant and are excreted together with Pb²⁺, typically via the kidney. The lead-monomensin complex is much less stable \((K = 10^{17.2})\) in 80% methanol/water) and has little solubility in an aqueous environment. Moreover, the complex easily crosses a phospholipid membrane, whereas Pb excretion occurs primarily via the colon. Thus, monemensin may act primarily by transporting Pb out of cells, where it eventually returns to the lumen of the gastrointestinal tract and is excreted by a normal transport mechanism, possibly involving the enterohoeptic circulation. Since monemensin is highly active as an ionophore for Na⁺ as well as for Pb²⁺, its presence will couple the gradients of these two cations across cell membranes, providing a driving force for Pb transport out of the cell. Overall, this potential mechanism suggests that the application of monemensin, together with a hydrophilic chelator, might be particularly effective at promoting the excretion of Pb from animals. That possibility is currently under investigation.

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Monensin Mediates a Rapid and Selective Transport of Pb\(^{2+}\): POSSIBLE APPLICATION OF MONENSIN FOR THE TREATMENT OF Pb\(^{2+}\) INTOXICATION

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