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The Effect of Modified Citrus Pectin on Urinary Excretion of Toxic Elements

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This study was undertaken to evaluate the effect of modified citrus pectin (MCP) on the urinary excretion of toxic elements in healthy individuals. MCP is a reduced molecular weight pectin (weight-average molar mass = 15400) that is mostly linear homogalacturonan with a 3.8% degree of esterification and approximately 10% rhamnogalacturonan II based on the presence of 2-keto-3-deoxy-octonic acid. Subjects ingested 15 g of MCP (PectaSol®, EcoNugenics® Inc., Santa Rosa, California 95407) each day for 5 days and 20 g on day 6. Twenty-four hour urine samples were collected on day 1 and day 6 for comparison with baseline. The urine samples were analysed for toxic and essential elements. In the first 24 h of MCP administration the urinary excretion of arsenic increased significantly (130%, $p < 0.05$). On day 6, urinary excretion was increased significantly for cadmium (150%, $p < 0.05$). In addition, lead showed a dramatic increase in excretion (560%, $p < 0.08$). This pilot trial provides the first evidence that oral administration of MCP increases significantly the urinary excretion of toxic metals in subjects with a 'normal' body load of metals. It is suggested that systemic chelation of toxic metals by MCP may in part be attributable to the presence of rhamnogalacturonan II, which has been shown previously to chelate metals. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: modified citrus pectin; rhamnogalacturonan; chelation; heavy metals; arsenic; PectaSol.

INTRODUCTION

Toxic metals such as arsenic, cadmium, lead and mercury disrupt normal endocrine, neurological, immune and/or other functions in the body (Carpenter, 2001). Poisoning with such metals is treated by the application of specific chelators such as EDTA (ethylenediaminetetraacetic acid), DMSA (meso-2,3-dimercaptosuccinic acid) or DMPS (sodium 2,3-dimercaptopropane-1-sulfonate) which bind the metals in the bloodstream and facilitate their removal via the urine and feces (Andersen and Aaseth, 2002). These treatments may reduce the levels of metals in the body, but they are also fraught with side effects such as redistributing metals to the brain or bone (Andersen and Aaseth, 2002; Cory-Slechta *et al.*, 1987), reducing critical minerals (Andersen and Aaseth, 2002), disturbing gastrointestinal function and causing skin rashes (Andersen and Aaseth, 2002). In addition, the application of chelation drugs can be ineffective due to a rebound back to pretreatment serum levels after treatment cessation. A milder chelation agent that could be administered orally would expand treatment options and reduce costs.

Pectin is a complex plant polysaccharide consisting of homogalacturonan, which is partially methyl esterified; rhamnogalacturonan I, consisting of alternating rhamnose and galacturonic acid residues with arabinan, galactan and/or arabinogalactan attached to the rhamnose residues; rhamnogalacturonan II, with a homogalacturonan backbone and complex branches containing neutral and acidic sugars including 2-keto-3-deoxyoctonic acid (KDO); and xylogalacturonan, with xylose attached to some of the galacturonic acid residues (Ridley *et al.*, 2001). This polysaccharide aggregates in solution to form networks of rods, segmented rods and kinked rods (Fishman *et al.*, 1993). Pectin is used as a gel-forming, viscosity-modifying, fiber food ingredient as well as for health-related non-food applications (Yamada, 1996).

Pectin demonstrates potential as an alternative to the conventional chelators. Orally administered pectin has been shown to decrease lead absorption (Paskins-Hurlburt *et al.*, 1977) and to reduce strontium bone and blood levels arising from an oral dose of this radioactive element (Waldron-Edward *et al.*, 1965). The ability of pectin to reduce absorption and the bioaccumulation of toxic metals is attributed to pectin binding the metals in the digestive tract and preventing their absorption while facilitating their elimination in the feces. This may also include metals that have been absorbed previously and have been excreted into the bile or undergone enterohepatic circulation (Niculescu *et al.*, 1969; Rowland *et al.*, 1986).

While pectin has been shown to be effective at reducing the absorption of lead and the lead body burden in heavily exposed individuals, studies investigating the effects of pectin in healthy individuals with normal lead exposure have not been done. Given the inability of researchers to find a 'safe' level of lead exposure

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(Canfield *et al.*, 2003), and the estimation that contemporary humans have levels 500 to 1000 times above those of pre-industrial humans (Flegal and Smith, 1992), research in people with 'normal' metal levels is warranted.

SUBJECTS AND METHODS

This research was conducted in accordance with the Declaration of Helsinki with the protocol approved by the University of California, Davis, Office of Human Research Protection. Eight subjects were recruited from a convenient sample and they provided written informed consent. All subjects were healthy adults. Subjects (two men and six women) ranged in age from 39 to 52 years old (mean = 45 standard deviation = 5).

Protocol. The subjects ingested 15 g of PectaSol® modified citrus pectin (EcoNugenics, Santa Rosa, CA) in three divided doses each day for 5 days and 20 g in four divided doses on day 6. A previous study showed good results using 15 g a day MCP in attenuating the progression of prostate cancer in men with biochemical relapse (Guess *et al.*, 2003). A reduction in heavy metals, in particular mercury, has been observed in men using MCP at 15 g a day long term as part of their prostate cancer treatment (Eliaz, 2004). Fifteen grams of MCP was selected for this pilot trial based on this clinical observation. An acute increase in urinary excretion was expected, but the magnitude of that increase was unknown. The increased amount of pectin ingested on day 6 was used in case the smaller amount did not give detectable changes in the urinary excretion of toxic metals. More than 20 g was not used in order to avoid potential intestinal discomfort from the high fiber content of the pectin. Prior to commencing the MCP ingestion, the subjects collected a 24 h urine sample as a baseline. Twenty four hour urine samples were collected on day 1 and day 6 for comparison.

Urine analysis. The urine samples were analysed by inductively coupled plasma mass spectrophotometer at a CLIA certified analytical laboratory (Doctor's Data, Inc. West Chicago, Illinois) for Al, Sb, As, Be, Bi, Cd, Ca, Cu, Fe, Mg, Hg, PB, Se, Tl, Th, Sn, U and Zn. Calibration was performed before each analysis batch and verified with an independent check sample (Lyphochek, BioRad, Irvine, CA).

Statistical analysis. Due to the small sample size a Shapiro-Wilk W test for normality was done on each set of data for each element. If $p < 0.05$ then the data were assumed normal and the data were analysed by the Student's *t*-test. If the Shapiro-Wilk test resulted in $p > 0.05$ the Wilcoxon signed-rank test (a non parametric test) was used to determine statistical significance.

MCP analysis. The galacturonic acid and KDO content was determined colorimetrically (Karkhanis *et al.*, 1978; Yoo *et al.*, 2003). The total neutral sugar carbohydrate content was determined by the phenol-sulfuric acid assay using a galactose standard (Manderson *et al.*, 2005). The degree of esterification was determined by gas chromatography (Lee *et al.*, 1975). Neutral monosaccharide analysis was performed following methanolysis using HPAEC-PAD with a CarboPAC PA20 column and an isocratic potassium hydroxide mobile phase (Manderson *et al.*, 2005). MCP was separated by high performance anion-exchange chromatography and pulsed amperometric detection (HPAEC-PAD) using a nonlinear potassium oxalate, pH 6 gradient and a CarboPac PA1 column (Hotchkiss and Hicks, 1990). A polygalacturonic acid hydrolysate was used for the oligogalacturonic acid degree of polymerization comparison.

The global molar mass of MCP was determined after dissolution in 0.05 M $NaNO_3$ by high performance size exclusion chromatography (HPSEC) with online refractive index, multi-angle laser light scattering (MALLS) and light scattering/viscometric (LS/V) detection (Fishman *et al.*, 2003). Two PL-Aquagel OH-60 and one PL-Aquagel OH-40 size exclusion columns were used in series. Other conditions included a sample concentration of 2 mg/mL, an injection volume of 200 µL and a flow rate of 0.7 mL/min using a 0.05 M $NaNO_3$ mobile phase.

RESULTS

Baseline urine samples were received for all subjects, however one subject missed the day 1 collection and a second person missed the day 6 collection. This resulted in a total of seven sets of data for each collection day. The results of urine analysis are summarized in Table 1. In the first 24 h of MCP administration the urinary excretion of arsenic increased 130% ($p < 0.05$). In this same period, the excretion of mercury increased 150% and cadmium increased 230% approaching significance with $p < 0.1$ (Table 1). On day 6, urinary excretion of cadmium (150%) increased significantly ($p < 0.05$). In addition, lead showed a dramatic increase in excretion (560% over baseline, $p < 0.1$) and the increase in tin excretion (130% approached significance with $p < 0.1$ (Table 1). No significant changes in the excretion of Al, Sb, Be, Bi, Ni, Pt, Tl, Th, U, Ca, Mg, Zn, Cu, Se and Fe were observed.

The galacturonic acid content of MCP was 74.6% (± 3.8), the degree of esterification was 3.8%, the KDO content was 0.46% (± 0.0) and the total neutral sugar carbohydrate content was 10.1% (± 0.7). The neutral monosaccharides in MCP (Table 2) were consistent with the neutral sugar-rich side chains in the rhamnogalacturonan region of pectin (Ridley *et al.*, 2001). MCP contains a series of oligogalacturonic acids with degree of polymerization values of at least 30 (Fig. 1). Peaks detected in the MCP chromatogram that did not agree with the oligogalacturonic acid retention times (under 20 min) may represent rhamnogalacturonan I oligosaccharides with galactose and/or arabinose attached to rhamnose residues, rhamnogalacturonan II oligosaccharides or possibly xylogalacturonan oligosaccharides. For comparison, the KDO content of rhamnogalacturonan II from red wine (gift from Alan Darvill, CCRC, University of Georgia, Athens, GA) was observed to be 4.8% (± 0.1).

The MCP weight average molar mass (M_w) and size (z average radius of gyration, R_{gz}) were determined by two procedures, the MALLS method and the LS/V

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Table 1. Urinary excretion of essential and toxic elements

Element		Al	Sb	As	Be	Bi	Cd	Ca	Cu	Fe	Pb
Day 0 (baseline)	Mean	7.3	0.12	26	<0.06	<0.02	0.54	180	0.01	0.3	0.38
μg/24 h	SD	11	0.13	14	0	0	0.23	100	0.003	0.04	0.42
Day 1 difference from	Mean	3.8	−0.02	9.4	0	0	0.35	2	0.004	0.14	−0.01
day 0 (μg/24 h)	SD	17	0.13	18	0	0	0.73	65	0.003	0.26	0.22
	p =			0.04			0.09				
Day 6 difference from	Mean	−0.52	0.04	5.3	0	0	0.16	40	0	0.06	0.82
day 0 (μg/24 h)	SD	2.0	0.16	22	0	0	0.20	290	0.002	0.17	1.1
	p =						0.02				0.08

Element		Mg	Hg	Ni	Pt	Se	Tl	Th	Sn	U	Zn
Day 0 (baseline)	Mean	81	1.5	3.3	<0.1	0.16	0.31	<0.06	0.70	0.01	0.30
μg/24 h	SD	37	0.53	2.7	0	0.08	0.13	0	0.52	0.02	0.19
Day 1 difference from	Mean	17	0.58	−0.62	0	0.04	0.03	0	0.11	0.01	0.09
day 0 (μg/24 h)	SD	37	1.3	1.15	0	0.01	0.05	0	0.28	0.02	0.15
	p =		0.08								
Day 6 difference from	Mean	88	0.72	−0.45	0	0.01	0.06	0.01	0.09	0.01	−0.04
day 0 (μg/24 h)	SD	200	1.3	0.95	0	0.04	0.21	0.03	0.32	0.02	0.14
	p =								0.06		

Table 2. Neutral monosaccharide composition of MCP (mol %)

Galactose	Rhamnose	Xylose	Fucose	Arabinose	Glucose
30.3	24.7	15.0	13.4	13.0	3.6

method (Fishman *et al.*, 2003). The MALLS method determines M_w and R_{gz} using the scattered light intensity at 14 angles, whereas the LS/V method determines these same properties from the intrinsic viscosity and the scattered light intensity at 90°. The MALLS method gave a value of 15 900 (± 400) for M_w and 12.1 (± 0) nm for R_{gz}, whereas the LS/V method gave 14800 (± 100) and 6.52 (± 0.06) nm for these same properties. The MCP molar mass calibration curve (A) was superimposed upon the refractive index curve (B) as a function of the column elution time in Fig. 2. Online viscosity detection revealed that the weight average intrinsic viscosity, $[\eta]_w$, of MCP was 0.398 (± 0.004) dL/g. The log of the intrinsic viscosity plotted against the log of the molar mass is known as the Mark-Houwink plot. The slope of this line (exponent *a*) was 1.45 ± 0.06 (data not shown).

DISCUSSION

This pilot trial provides the first evidence that moderate amounts of MCP significantly increased the urinary excretion of toxic metals, but not essential minerals in subjects with a 'normal' body load of metals. The urinary excretion of the known neuro- and immunotoxins: arsenic, mercury, cadmium and lead, increased within 1–6 days of MCP treatment. The ability to rapidly detect significant changes in urinary excretion of metals with a mild treatment such as MCP is encouraging, especially given the small number of heterogeneous subjects included in the study. In addition, these results suggest that MCP administration and subsequent urine collections could be developed into a mild, non-invasive test to assess the relative load of toxic metals in human subjects. This application would require further refinement of the methods and demonstration that urinary excretion is correlated to body load.

HPSEC studies of commercial high methoxy citrus pectin revealed that the M_w, R_{gz}, $[\eta]_w$ and *a* are 232 000, 36 nm, 6.41 dL/g and 0.65, respectively (Fishman *et al.*, 2001). Except for *a*, these values are appreciably higher than those found for MCP. In that same article it was demonstrated that *a* increases as the molar mass decreases. The linearity of the Mark-Houwink plot (data not shown) and the higher value of *a* for MCP compared with commercial citrus pectin (i.e. 1.45 against 0.65) indicated that MCP had a more uniform and linear shape when compared with commercial citrus pectin.

The size and composition of MCP is consistent with a reduced molecular weight and debranched pectin consisting of a homogalacturonan backbone with little methyl esterification. This type of structure, with a series of negatively charged galacturonic acid residues, has excellent potential for cation chelation (el-Zoghbi and Sitohy, 2001; Kohn, 1982). Since rhamnogalactuonan II is the only plant polysaccharide to contain KDO, MCP consists of approximately 10% rhamnogalacturonan II. The backbone of red wine rhamnogalacturonan II (dimer molecular weight = 10 000) consists of up to DP 15 oligogalacturonic acids (Pellerin *et al.*, 1996), well within the oligogalacturonic acid range detected for MCP.

The increased urinary excretion of metals observed when MCP is administered is probably due to direct chelation in the bloodstream by the low molecular weight pectin containing rhamnogalacturonan II and subsequent elimination of the metal–pectin complex in the urine. Rhamnogalacturonan II dimers (borate diester crosslinks between apiosyl residues) are well

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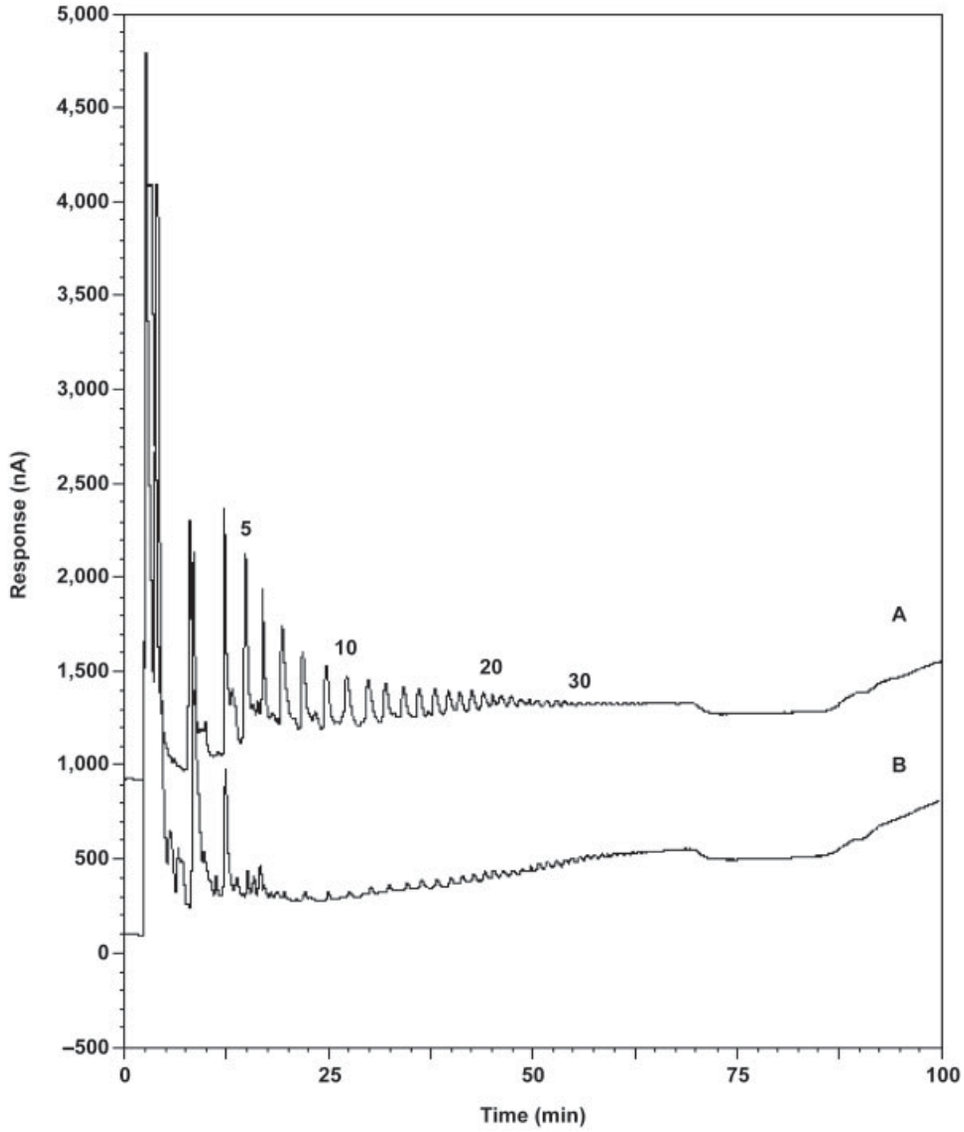

Figure 1. Oligosaccharide analysis of MCP by HPAEC-PAD. A polygalacturonic acid hydrolysate (A) was compared with MCP (B). The oligogalacturonic acid degree of polymerization is indicated above the peaks. A CarboPac PA1 column and a nonlinear potassium oxalate, pH 6 gradient mobile phase was used.

known as metal cation chelators (O'Neill *et al.*, 1996; Tahiri *et al.*, 2000). Native sized pectin persists in the gut until it is completely degraded by bacterial fermentation in the colon. However, the effectiveness of orally administered MCP in prostate cancer suggests absorption prior to colonic breakdown (Guess *et al.*, 2003). Supporting this hypothesis is evidence of a galactan side chain fragment of rhamnogalacturonan I found in follicles of Peyer's patches and a liver homogenate 1 week after oral administration of rhamnogalacturonan I to mice (Sakurai *et al.*, 1996). Additionally, linear polygalacturonides adhere optimally to colonic epithelial membrane mucins (Schmidgall and Hensel, 2002) and deesterified pectin penetrates deeper into colonic epithelial crypts (Liu *et al.*, 2005) compared with more highly esterifed or branched pectins in *ex vivo* assays. The characteristics of the MCP used in this study support uptake by gastrointestinal associated lymphoid tissue, however, the exact mechanism of gastrointestinal uptake for MCP remains to be determined.

The changes in urinary excretion observed in this study were modest and longer, larger studies are needed to determine if these small changes, sustained over an extended period could have a relevant clinical effect. The potential therapeutic importance of MCP as a mild chelator that could be used on an ongoing basis is immediately evident in light of the evidence that levels of lead previously considered safe have now been shown to result in significant differences in intelligence in children (Canfield *et al.*, 2003) and conventional chelation therapy seems ineffective at both reducing serum lead and neuropsychological impairments in children.

Our results are also in agreement with previously reported binding trends. The metal binding properties of pectin have been investigated *in vitro* and the relative affinity for a variety of minerals and toxic metals has been presented by various research groups (Table 3). We observed the urinary excretion of essential elements (such as zinc, calcium and magnesium) was not affected, in concordance with the higher binding affinity that pectin and pectin monomers

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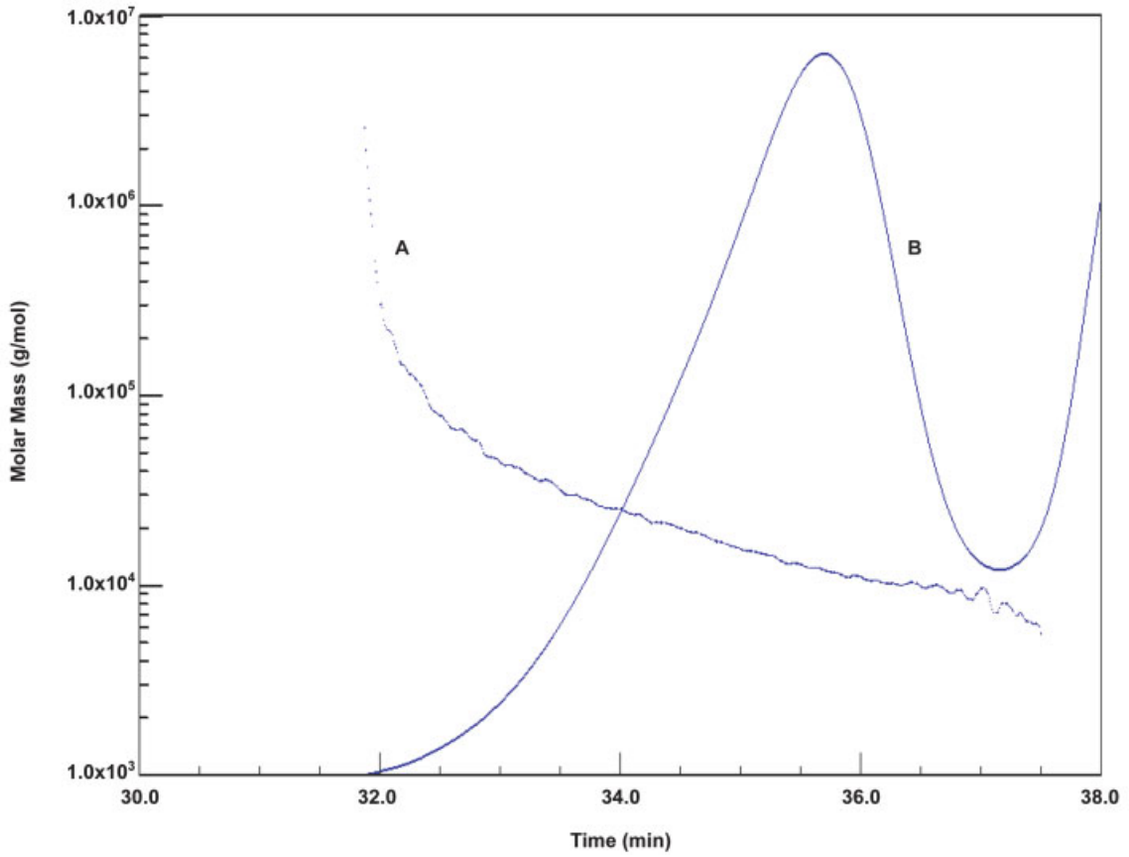

Figure 2. Characterization of MCP by HPSEC with online multi-angle laser light scattering and differential refractive index detection. Plot of MCP molar mass against elution time (A) superimposed on plot of differential refractive index response against elution time (B).

Table 3. Relative binding affinities of pectin and select metals

Relative binding affinities	Reference
Pb >> Cu > Co > Ni >> Zn > Cd	(Kartel *et al.*, 1999)
Pb ≃ Cu > Cd > Ni > Ba > Zn > Ca > Sr > Co > Mn > Mg	(Haug and Smidsrod, 1970)
Sr > Pb > Ni > Cd > Mn > Cu > Ba > Co > Zn > Fe > Mg > Ca > Cr > Hg	(Waldron-Edward *et al.*, 1965)
Pb > Ba > Cd > Sr > Zn > Cu > Co > Ni > Fe > Hg > Cr > Mn > Mg in the presence of calcium	
Pb >> Ca ≃ Sr	(Braudo *et al.*, 1996)

(galacturonic acid) have for toxic metals over essential minerals (Braudo *et al.*, 1996; Kohn, 1982; Paskins-Hurlburt *et al.*, 1977). Additionally, the cation chelation by rhamnogalacturonan II dimers is specific for Pb, Ba, Sr, La, Eu, Ce, Pr and Nd, whereas essential cations such as Ca, Mg, Fe, Zn and Cu are not bound (O'Neill *et al.*, 1996; Tahiri *et al.*, 2000). Although some metals (cadmium and strontium) vary widely in their placement in the relative binding affinity series (Table 3), lead consistently exhibits the highest affinity for pectin.

In accordance with these trends, elevated urinary excretion of cadmium and lead was observed but no changes occurred in the urinary excretion of cations with lower pectin affinity. Although mercury, arsenic and tin were not included in previous affinity studies we would expect them to exhibit strong affinity to pectin, based on the relative binding trends from existing research. In general, elements to the right on the periodic table and those lower within the same series in the periodic table tend to form the strongest complexes with polyuronates.

In conclusion, oral administration of modified citrus pectin (molecular weight 15400; DE 3.8%) resulted in significant increases in the urinary excretion of arsenic and cadmium. Mercury and lead also demonstrated increased excretion approaching significance. This preliminary work suggests that the nutritional supplement, MCP, may assist in the elimination of toxic elements from the body via chelating the metals in the bloodstream and elimination in the urine. A non-toxic chelating agent, such as MCP, that potentially could be used safely for prolonged periods would be an important treatment alternative for children exposed to lead on an ongoing basis and for people in which conventional chelators are contraindicated. Additional larger randomized placebo controlled studies involving individuals with toxic element burden and/or comparing MCP to regular pectin or conventional chelating drugs are warranted.

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