Petrographic and geochemic evaluation of equine enteroliths

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Petrographic and geochemic evaluation of equine enteroliths

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Objective—To characterize the texture, mineralogic features, and chemical features of enteroliths obtained from horses.

Sample Population—Enteroliths from 13 horses with colic.

Procedure—Enteroliths were harvested from 13 horses that underwent ventral midline celiotomy for treatment of colic or necropsy because of colonic obstruction and rupture caused by enteroliths. Dietary and environmental history were determined via questionnaires or evaluation of medical records. In 7 horses that underwent surgical treatment for enterolithiasis, samples of colonic contents were obtained via an enterotomy in the pelvic flexure. Colonic concentrations of magnesium (Mg), phosphorus (P), sulfur (S), sodium (Na), calcium (Ca), and potassium (K) were determined. Enteroliths were analyzed via electron microprobe analysis and X-ray diffraction.

Results—Enteroliths varied widely regarding degree of porosity, presence and distribution of radiating texture, and composition and size of the central nidus. A distinct concentric banding was identifiable in all enteroliths. Struvite was the predominant component of all enteroliths, although Mg vivianite was identified in 5 enteroliths, and there were variable quantities of Na, S, K, and Ca in the struvite within enteroliths. Despite an abundance of Ca in colonic fluids, Mg-phosphate minerals were preferentially formed, compared with Ca-phosphates (apatite), in equine enteroliths.

Conclusions and Clinical Relevance—Enteroliths comprise 2 major Mg phosphates: struvite and Mg vivianite. There is wide variability in macrotexture and ionic concentrations between and within enteroliths. (*Am J Vet Res* 2001;62:350–358)

E nteroliths are intestinal calculi that form in the ampulla coli of the right dorsal colon of horses and result in colic because of partial or complete obstruction of the right dorsal, transverse, or descending colon.[1-3] Equine enterolithiasis occurs worldwide; however, the disease is unusually prevalent in some regions, such as California. At the University of California, Davis, horses with enterolithiasis represent 27.5% of the surgical colic caseload, and enteroliths have become the leading cause of colic requiring surgical correction.[1]

Although the specific pathogenesis of enterolithiasis is unknown, hypotheses for factors contributing to enterolith formation include excessive concentrations of dietary **magnesium (Mg)**, **phosphorus (P)**, and nitrogen, the presence of nidi, alkaline colonic contents, breed, relative hypomotility of the right dorsal colon, and cation content of the water supply.[1-7] Probable risk factors for development of enteroliths include high amounts of dietary alfalfa and lack of daily pasture grazing.[a] Results of early studies indicate that in vivo dissolution of enteroliths may occur, and alterations in intestinal pH may play an integral role in enterolith formation and dissolution.[7,8] This pH dependency is consistent with findings regarding struvite uroliths in cats.[9]

Equine enteroliths are generally smooth-surfaced spheroidal or tetrahedral phosphatic stones, but wide variations in shape, texture, and size may occur. Although the bulk chemical composition of enteroliths was first determined > 150 years ago,[10] details of petrographic and geochemic data on a series of enteroliths have yet to be described. The purpose of the study reported here was to systematically characterize the texture, mineralogic features, and chemical features of a collection of enteroliths.

Materials and Methods

Collection of specimens—Fourteen enteroliths were harvested from 13 horses that lived in various geographic locations. Specimens were obtained during celiotomy for treatment of colic or during postmortem examination after rupture of the gastrointestinal tract attributable to enterolith obstruction. Enteroliths were the primary source of colonic obstruction in 12 of 13 horses. More than 1 enterolith was identified in 8 of 13 horses. In 1 horse (horse 3), multiple small enteroliths of similar size and morphology were obtained at the time of enterotomy for treatment of sand colic, and 2 were randomly selected for analysis. Because of the small size of these enteroliths, results of mineral analysis of both enteroliths were pooled. For horses with > 1 large enterolith, gross morphologic characteristics were comparable, and a single enterolith was randomly selected for analysis. A history was obtained for each horse via owner questionnaire or the medical record regarding present diet, location, and any alterations in diet or location during a 5-year period. For those horses admitted to the University of California, Davis, Veterinary Medical Teaching Hospital, samples of colonic ingesta were also obtained. Enteroliths were washed with tap water, dried, and stored at 20 C until analysis. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

Colonic ingesta analysis—Specimens of ingesta from the ascending colon were obtained via enterotomy in the
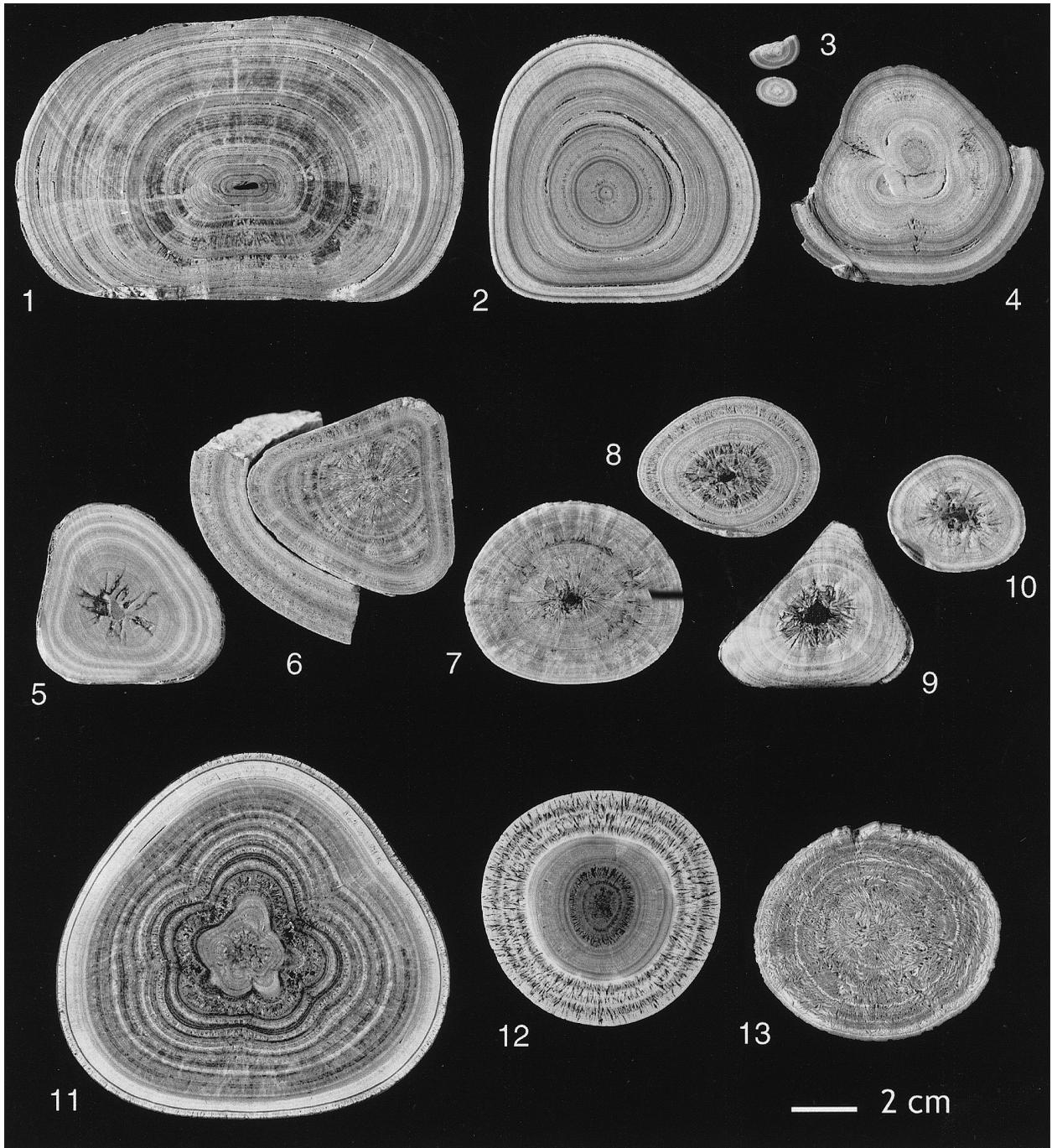
Figure 1—Photograph of polished slabs of equine enteroliths revealing macrotexture. Specimens are arranged in 3 groups, according to textural types. Top—Low-porosity concentrically banded enteroliths (1–4). Center—High-porosity enteroliths with radiating textures in their cores (5–10). Bottom—Mixed-textural types (11–13).

pelvic flexure. Wet samples were stored at –70 C for further analysis. **Sulfur (S)**, **sodium (Na)**, **calcium (Ca)**, **potassium (K)**, **Mg, and P** concentrations were determined via inductively coupled argon emission spectroscopy, using hydride generation.[b]

Enterolith specimens—Enteroliths were sectioned into 2 equal halves with a band saw, which allowed identification of the central nidus. The cut surface was polished and photographed. Size, color, shape, and physical features of the internal and external surfaces of the enteroliths were documented. Features of internal architecture, including porosity,

textural type (concentric banding and radiate texture) and crystal size (fine- or coarse-grained), were described. Primary porosity consisted of open spaces resulting from the original arrangement and size of crystals during calculus formation, whereas secondary porosity represented pore spaces created after crystal formation as a result of dissolution.[11]

X-ray diffraction—Fragments of enteroliths from core and rim regions were prepared,[12] and the resulting powder was mounted on a zero-background quartz crystal slide. Mineralogic composition was determined by use of an X-ray diffractometer[c] equipped with a copper target operated at
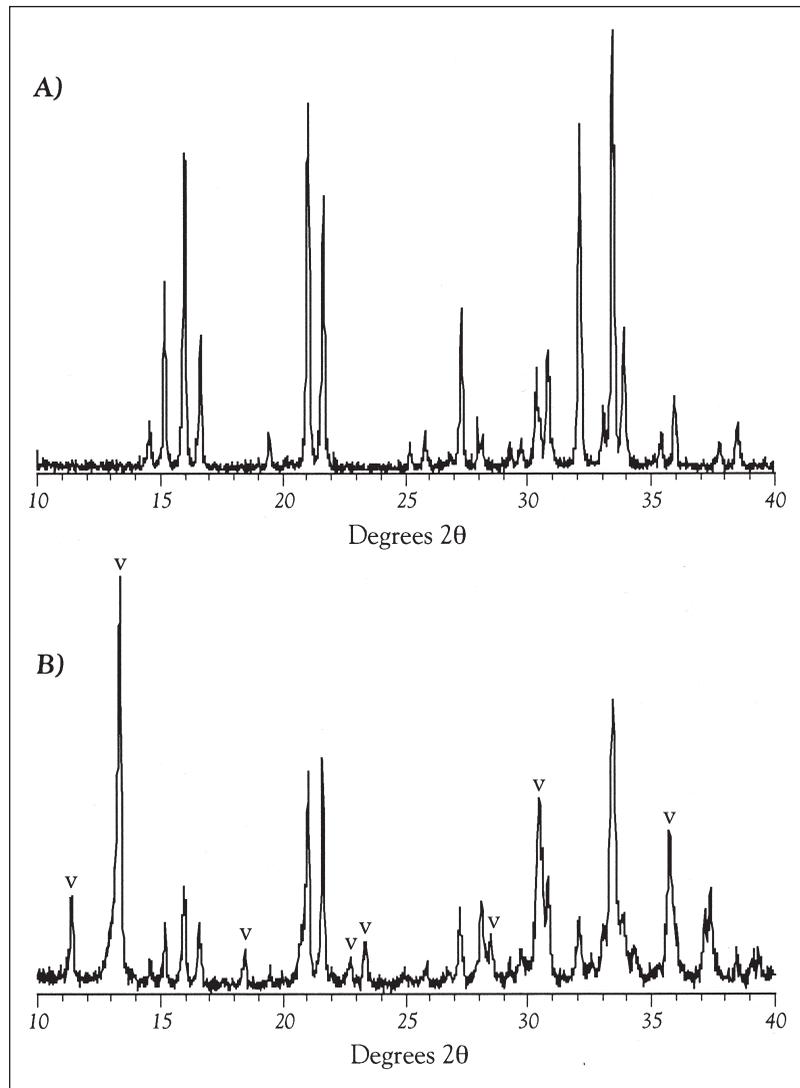
Figure 2—X-ray diffraction patterns of material removed from 2 equine enteroliths. A—Pure struvite from the core region. B—Mixed struvite and vivianite (v) from the rim region of a different enterolith. Notice that the X-ray diffraction pattern in B has all the peaks (corresponding to struvite) seen in A as well as additional peaks that indicate detection of vivianite.

45 keV and 45 mA.[13] Specimens were scanned at a rate of 10°/min between 10° and 50° of 2θ to identify the diffraction lines as peaks above the background readings.[13,14] X-ray diffraction patterns of the enterolith specimens were compared with standard patterns to identify minerals.

Preparation of enterolith slabs—Enteroliths were cut into billets for preparation of doubly polished thin sections.[12] Billets were dried for several hours on a hot plate at temperatures ≤ 30 C, because at substantially higher temperatures, struvite will partially dehydrate to dittmarite.[15] During drying, the billets were placed on aluminum foil and liberally coated with a high-vacuum epoxide that set up slowly. The epoxy minimized the effects of crystal breakage and grain plucking, which can alter true texture and porosity of specimens. The enterolith thin sections were polished with fixed diamond abrasives to avoid driving loose abrasive material into fractures and pore spaces.[15]

Electron probe microanalysis of enteroliths (microprobe)—Polished sections were examined by use of an elec-tron microprobe analyzer[d] equipped with a high-speed **back-scattered electron (BSE)** detector, an energy dispersive spectrometer, and 3 wavelength dispersive X-ray spectrometers.[16-19] By use of the BSE mode, struvite crystals appeared gray, vivianite appeared as a finely granular darker gray crystal in porous regions, and open spaces between crystals appeared black (epoxide mounting medium). Scanning electron micrographs were used to document the observed textures, and matching X-ray dot maps were prepared to compare with primary textural features. Qualitative wavelength scans were used to identify which elements were in detectable concentrations. Quantitative analyses and X-ray dot maps were performed to determine the Mg, P, K, Ca, Na, and S content and distribution within each specimen, as described.[14] Analytic parameters as well as peak and background intensities were determined for each of the analyte lines (ie, Mg Kα [X-ray emission line associated with a K-series electron vacancy], P Kα, S Kα, K Kα, and Ca Kα). Peak minus background intensities of analyte lines for specimens, relative to standards, were quantified by use of standard matrix correction techniques for atomic number, absorption, and fluorescence effects.[19] In 1 enterolith spec-
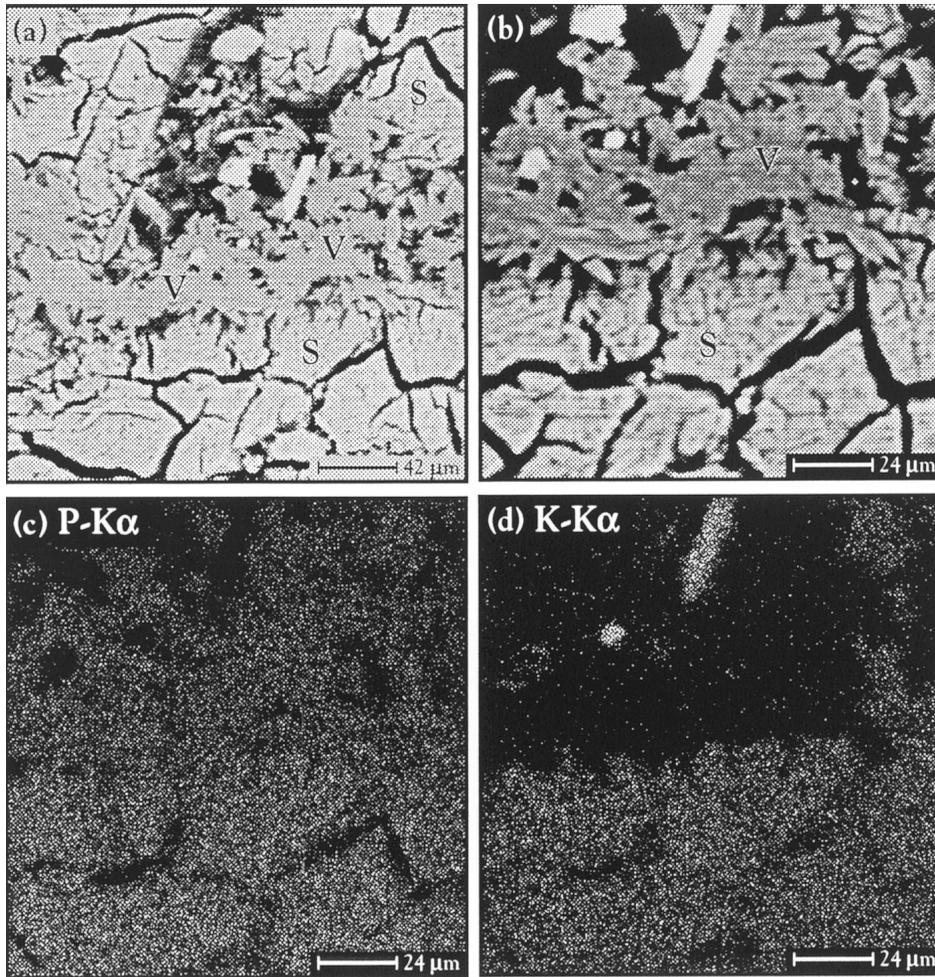
Figure 3—Back-scattered electron (BSE) and X-ray dot map images depicting microtextural and chemical relationships between primary struvite (S) and secondary vivianite (V) crystals within enteroliths. (a)—A BSE image depicting vein of vivianite crystals (dark material in center) that cut pre-existing struvite (light material). The bright crystals in the center of the photo are potassium (K)-rich mica. (b)—Close-up view of area shown in (a). (c)—Phosphorous (P) Kα X-ray (X-ray emission line associated with a K-series electron vacancy) dot map of region shown in (b). (d)—Potassium Kα X-ray dot map of area shown in (b). Notice that struvite contains K, but vivianite does not. Both minerals contain phosphorous.
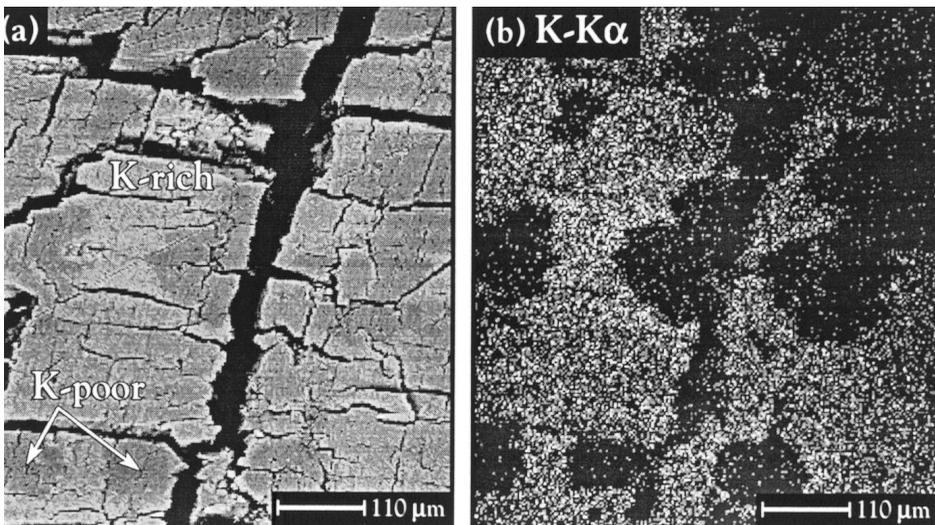

Figure 4—A BSE image (a) and K-Kα X-ray dot map (b) depicting the spatial distribution of K within struvite in an equine enterolith. In the BSE image, the K-rich regions are lightly shaded. Notice that there is no evidence of vivianite crystal formation.
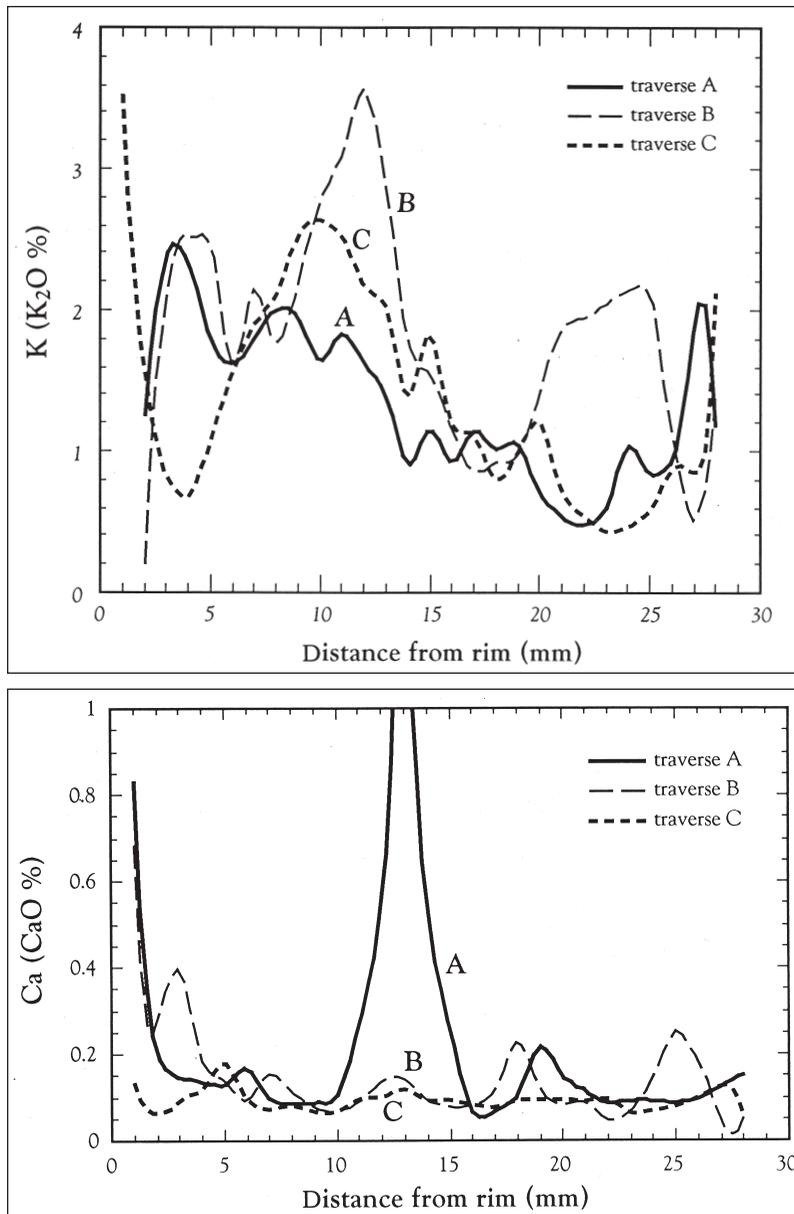
Figure 5—Concentrations of K (expressed as K_2O weight %; upper graph) and calcium (Ca; expressed as CaO weight %; lower graph) in equine enteroliths, determined by use of electron microprobe spot analyses along 3 linear analytic traverses (marked A, B, and C) that started at the rim and ended near the nidus region of the specimen. The linear traverses are parallel to each other but are offset from each other by approximately 1 cm.

imen, concentrations of K and Ca were determined by use of electron microprobe spot analyses along 3 linear analytic traverses starting at the rim and ending near the nidus. The linear traverses were parallel to each other and 1 cm apart. Concentration profiles for K and Ca were evaluated within each traverse to determine whether compositional changes follow a pattern that may represent alterations in the intestinal milieu during enterolith formation.

Results

Enteroliths were obtained from 13 horses from Arizona, Idaho, and southern, central, and northern California. Mean duration of enterolith storage prior to analysis was 18 months. Alfalfa hay constituted most of the diet (median, 100%; range, 50 to 100%). There were no dietary changes or changes in geographic location in the 5 years prior to enterolith removal.

Physical characteristics—Enterolith diameter ranged from 1 to 14 cm. A central nidus was identified in most enter-oliths, although there was wide variability with respect to size and type. All nidi in this series were comprised of rock, and all were < 1 cm in diameter. Grains of sandstone, metabasalt, granite, chert, serpentinite, and basalt were identified as nidi in some enteroliths, and a discreet nidus was not identifiable in 4 enteroliths. Although 1 enterolith lacked a discernible central nidus, mineral grains were identifiable in cracks near the central region. Shape was spherical
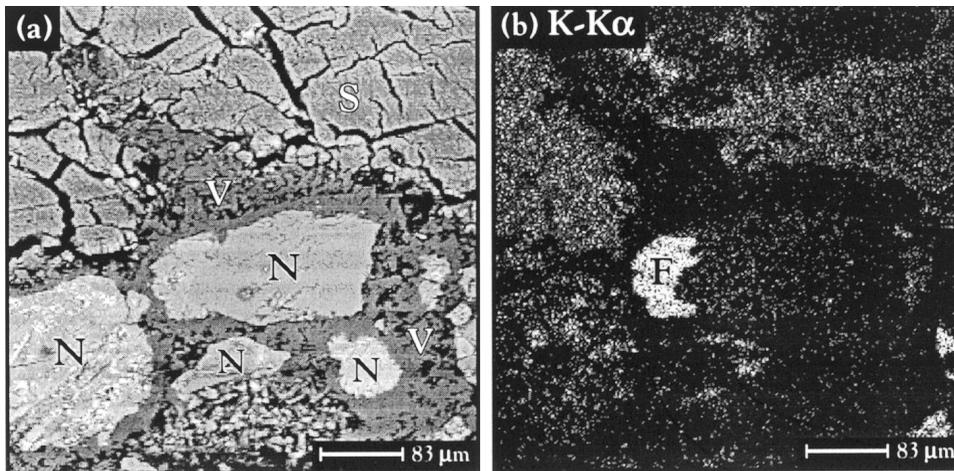
Figure 6—A BSE image (a) and K-Kα X-ray dot map (b) depicting textural and chemical relations within the nidus region of equine enteroliths. The nidus is a stone that has broken into many small mineral grain nidi (N) surrounded by vivianite (V). Lightly shaded material containing fissures is struvite (S). Notice that struvite contains K, but vivianite does not. The K-rich grain in the nidus is a K feldspar (F).
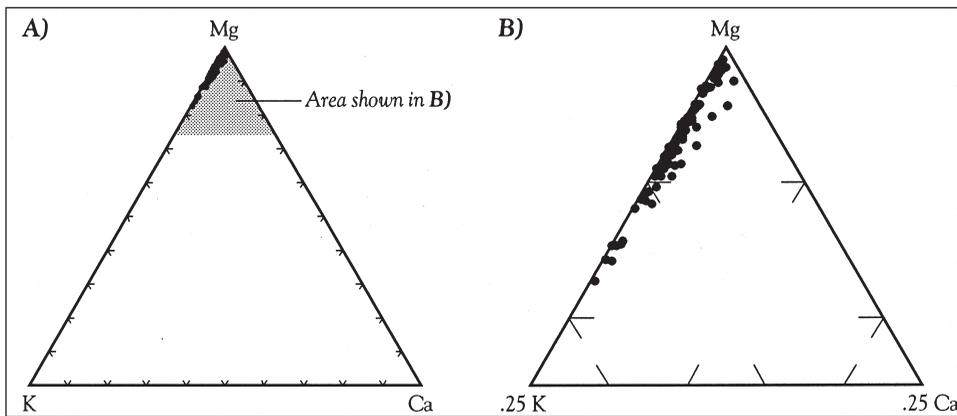

Figure 7—Ternary diagram of relative mole percentages of magnesium (Mg), K, and Ca in struvite crystals in equine enteroliths. A—Apices represent 100% for the respective elements; tick marks represent increments of 10% difference in relative mole percentage. B—Detail of Mg-rich portion of A; apices represent 100% for Mg and 25% for K and Ca; tick marks represent increments of 10% difference in relative mole percentage. Notice that struvites contain a wide range in K content and more restricted range in Ca content. For example, in A, a dot directly on the Mg apex represents a pure struvite composition ($MgNH_4PO_4 \cdot 6H_2O$). A dot 1 tick mark down from the Mg apex along the Mg-K sideline of the triangle has a composition of ($Mg_{0.9}K_{0.1})NH_4PO_4 \cdot 6H_2O$.

Table 1—Elemental composition (mean [range]) of struvite (n = 13) and corresponding colonic fluids (7) from 13 horses with enteroliths

Element	Struvite	Colonic fluid
Na	0.02 (0.0–0.05)	1.24 (0.67–2.33)
S	0.01 (0.01–0.02)	0.25 (0.15–0.43)
K	1.47 (0.71–2.51)	0.86 (0.25–1.63)
Ca	0.13 (0.07–0.19)	0.80 (0.44–1.72)
Mg	13.0 (13.0)	0.45 (0.24–1.09)
P	22.0 (22.0)	0.85 (0.39–1.20)
N	NA	0.38 (0.20–0.68)
pH	NA	7.0 (6.7–7.3)

Na = Sodium. S = Sulfur. K = Potassium. Ca = Calcium. Mg = Magnesium. P = Phosphorus. N = Nitrogen. NA = Not available.

or tetrahedral. Enteroliths with a distinct tetrahedral shape were derived from horses with > 1 enterolith at the time of surgical exploration or necropsy, although spherical enteroliths were also found in some of these horses. Enteroliths were informally arranged into 3 groups on the basis of macro-textural characteristics (**Fig 1**). Porosity and presence or absence of radiate texture in the core region distinguished these textural types. Enteroliths were classified into a low-porosity concentrically ban-ded type (n = 4), a high-porosity type with radiating textures within the core region (6), and a mixed-textural type with characteristics of low- and high-porosity types (3). Crystal size was variable within and between enteroliths and was not associated with particular structural characteristics.

Mineralogic features—X-ray diffraction patterns revealed that enteroliths contained either struvite (magnesium ammonium phosphate hexahydrate [$MgNH_4PO_4 \cdot 6H_2O$]) or a mixture of struvite and the mineral Mg vivianite ($Mg_3 [PO_4]_2 \cdot 8H_2O$; **Fig 2**). Vivianite was identified in 5 enteroliths and could be found within the core or rim region of the enterolith. This mineral was recog-
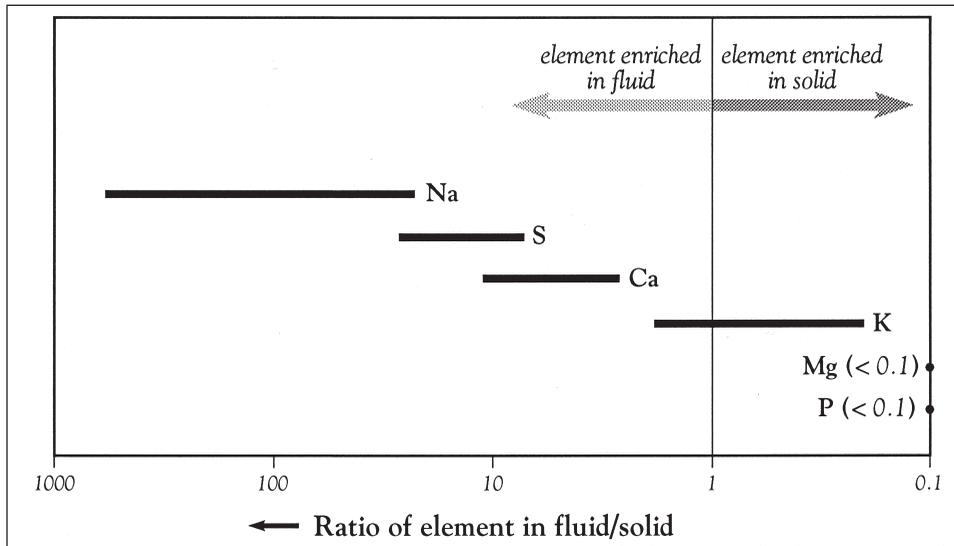
Figure 8—Plot depicting range of concentrations of selected ions (Na, S, Ca, K, Mg, and P) in colonic fluids, relative to their concentrations in struvite crystals in equine enteroliths. The numeral 1 represents equal concentrations in the fluid and solid phases. The fluid/solid ratios for Mg and P are < 0.1.

nized in 4 of 6 high-porosity radiate-textured enteroliths versus 1 of 4 low-porosity banded-texture enteroliths. It was not identified in examined sections of the mixed-textural type enteroliths.

Porosity—Secondary porosity was commonly identified in the enteroliths with radiate textures. The radially textured pattern was always superimposed on original fine concentric laminations identical to those in the outer zone, and vivianite crystals were identified in pore spaces between struvite crystals (Fig 3).

Trace elements—Elemental concentrations, determined by use of electron microprobe analysis techniques, indicated variable quantities of K within struvite crystals in addition to the Mg and P peaks (Table 1). Evaluation of BSE micrographs and corresponding X-ray dot maps revealed subtle morphologic differences in struvite composition related to variations in K content (Fig 4). Potassium and Ca contents were evaluated along 3 linear analytic traverses that extended from rim to central nidus in 1 enterolith, and were found to lack a distinct pattern of distribution (Fig 5). Analysis of K and P Kα X-ray dot map images of regions that contained vivianite revealed K-rich regions within struvite and absence of K in vivianite. In contrast, P was in nearly equal concentrations within struvite and vivianite (Fig 3 and 6).

The proportions of Mg, P, and Ca in struvite crystals, as determined by quantitative electron microprobe analyses, were plotted on a ternary diagram in terms of relative mole percentages (Fig 7). Observation of detail of the Mg-rich portion of the diagram revealed the wide range in K content and relatively restricted range in Ca within struvite in enteroliths.

Comparison of concentrations of selected ions (Na, S, Ca, K, Mg, and P) in colonic contents was examined relative to their concentrations within struvite from the corresponding enteroliths (Table 1). Ratios of the concentration of elements in colonic con-

tents (fluid phase) relative to concentrations within enteroliths (solid phase) were plotted (Fig 8). Elements abundant in colonic fluid (Na, S, Ca) were detected in small quantities within enteroliths. Conversely, elements abundant in enteroliths (Mg, P) were detected in proportionately lower concentrations in their surrounding colonic fluids. Despite the abundance of Ca in colonic fluids, Mg-phosphate minerals were in greater concentrations than were Ca-phosphate minerals (apatite) in the enteroliths.

Discussion

Identification of magnesium ammonium phosphate ($NH_3MgPO_4 \cdot 6H_2O$) as the predominant component of enteroliths is consistent with previous reports.[3-6,20,21] The enterolith appears to grow outward from a central nidus that commonly forms from rock fragments or disaggregated mineral grains (Fig 6). The composition of the nidus appears to be highly variable, and a distinct nidus was not identifiable in 4 enteroliths. Absence of an apparent nidus was particularly evident in solitary enteroliths that were classified as mixed-textural types. This may be the result of a small organic or inorganic focus that was undetectable because of its size or location of sectioning of the enterolith. Reported nidi include mineral grains as well as feed material, plastic, rope, horse hair, cloth, and metallic objects such as wire or nails.[2-4] Under the appropriate conditions, struvite crystalline layers are constructed, grow outward from central nidi, and form concentric multilamellar bands. This banding pattern differs in texture and origin from that observed in struvite urinary calculi in dogs and cats.[11,14,15] Differences may reflect the rate of nucleation and crystal growth or degree of supersaturation in the surrounding aqueous medium. Low degrees of supersaturation favor coarse struvite crystals and slow growth rate, whereas high degrees of supersaturation, typical of the cores of many struvite-forming urinary calculi, favor high nucleation to growth rates ratios and, consequently, fine-grained sizes.[14,15] The alterations in grain size

observed in the equine enteroliths reported here may reflect the degree of struvite saturation within the colon and the rate of growth of the enteroliths.

Precipitation of struvite is generally attributed to Mg^{2+} supersaturation, presence of NH_4^+ and $PO_4^{3-},$ and an alkaline pH.[22,23] These findings are consistent with a recent preliminary report of higher concentrations of cations, lower percentage dry matter, and higher pH in the ascending colon of horses with enteroliths, compared with control horses.[a] These conditions, combined with the natural relative hypomotility within the right dorsal colon,[24] likely contribute to the optimal environment for precipitation of struvite. Magnesium concentration and colonic pH in horses appear to be diet-dependent, with Mg-rich alfalfa hay being the most commonly implicated dietary factor.[3-8,a] Despite its alkalinizing effects and high Mg content, alfalfa hay cannot be solely responsible for the formation of enteroliths, because most horses fed an alfalfa diet do not develop enteroliths. Other factors influencing intestinal pH or mineral content within the colon must also be considered, such as undetermined genetic factors, diet, bacterial flora, innate deficiencies, or buffering capacity and pH of the water supply.[1] The diets of the horses included in the study reported here consisted primarily of alfalfa hay, supporting previous suggestions of alfalfa as a risk factor for development of enterolithiasis. Because of this lack of variation in diet, any correlation between diet and alteration in mineral composition of enteroliths could not be evaluated.

Precipitation of struvite occurs together with apatite (Ca phosphate) in canine and feline urinary calculi, and the Mg:Ca in solutions determines which phosphate mineral will form.[25] Apatite and struvite may be precipitated almost simultaneously when conditions are favorable for apatite precipitation.[14] In contrast, this population of equine enteroliths contained a highly restricted range of relative mole percentages for Ca despite having a higher concentration of Ca and a higher fluid-to-solid ratio of Ca, compared with Mg, in the ascending colon. Substitution of Ca for Mg to form apatite was not observed. Inhibition of incorporation of Ca may be a result of the presence of other ions. Low concentrations of pyrophosphate may cause suppression of apatite crystal growth, and Mg disturbs crystallization of apatite.[25,26] In addition, the substitution of various ions (eg, K^+ or Na^+ for Ca^{2+}; CO_3^{2-} for PO_4^{3-}; Cl^- or F^- for OH^-) may cause alterations in the physicochemical properties of apatite.[25] A high concentration of K was observed within the struvite. Although not observed in this population of enteroliths, increased Ca incorporation into the ammonium phosphate of enteroliths has been observed in other geographic locations.[3] These observed differences may reflect variation in concentrations of trace elements in the soil, water supply, or feed. However, observations on 1 enterolith examined along 3 linear analytic traverses for concentrations of K and Ca did not reveal a correlation with growth banding. Such a pattern may be anticipated if trace element variations were attributable to chronologic variations in feed or water supply. Results of X-ray dot map imaging were also supportive of an unusual pattern of distribution of K in struvite (Fig 4).

The collection of enteroliths we studied was obtained from horses from the western United States. It is possible that additional evaluation of enteroliths obtained from horses in the eastern United States or other regions of the world may reveal differences in mineralogic features related to various unique geographic and dietary differences. For horses with > 1 enterolith, a sample was randomly selected for analysis. Although differences in petrographic and chemical composition of enteroliths obtained from the same animal may exist, the observed similarities in color and gross morphology of these enteroliths suggest similarities in composition.

A unique feature identified in several enteroliths was an apparent mineralogic transformation of struvite to Mg vivianite. A radially textured pattern was always superimposed on original fine concentric laminations, and pore spaces likely represented chemical dissolution within the core region of the enterolith. Vivianite crystals were identified filling pore spaces between struvite crystals. On the basis of morphologic characteristics and evaluation of back-scattered electron images, vivianite is considered to have developed secondarily to the original struvite mineral (Fig 3 and 6). Vivianite was identified in only 5 of 14 enteroliths, although specimens obtained for analysis represent between 5 and 30% of the entire enterolith structure. It is possible that localized or low concentrations of vivianite existed in other portions of the enteroliths. Although there are several potential mechanisms for conversion of struvite to vivianite and creation of secondary porosity within the enterolith, all potential reactions must entail dehydration and deammonification (ie, decreasing $[NH_4]^+$ activity or decreasing pH).[15] Assuming the conservation of phosphorus, 1 possible mechanism could be explained by the following reaction:

$$2NH_4MgPO_4 \cdot 6H_2O + Mg^{2+} = Mg_3(PO_4)_2 \cdot 8H_2O + 2(NH_4)^+ + 4H_2O^{15}$$
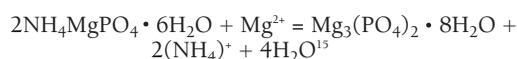
By use of this reaction, 490.6 g of struvite per formula unit (at a density of 1.71 g/cm^3) may convert to 406.9 g of Mg vivianite per formula unit (at a density of 2.23 g/cm^3).[27] Volumetrically, 287 cm^3 of struvite converts to 182 cm^3 of Mg vivianite, resulting in a 36% net increase in porosity. Because struvite precipitation is influenced by the action of ammonia-fixing bacteria in the urinary tract,[28] the bacterial mediation of struvite precipitation in equine enteroliths seems likely. Transformation of struvite to vivianite in enteroliths may be related to the death or cessation of metabolic activity of these bacteria within the core of the enterolith after the nitrogen feed source is depleted.[15] The presence of potential nitrogen feed sources such as cellulose fibers and organic material within pore spaces of enteroliths has been described.[15]

Results of our study revealed variation in textural type that may represent transformation of struvite to the mineral vivianite, resulting in secondary porosity within the internal architecture of the enterolith. Struvite in enteroliths was also found to contain high concentrations of K (up to 5 %, by weight) that are not homogeneously distributed. A linear correlation

between the concentrations of cations in colonic fluids and in the phosphate minerals was not identified, and no distinct differences were observed in the population of enteroliths studied. Despite colonic fluids that contained greater quantities of Ca than Mg in all horses evaluated, apatite (Ca-phosphate) minerals were not found in our equine enteroliths. In studies that evaluated the features of canine and feline urinary calculi, struvite calculi were more amenable to dissolution than were apatite calculi.[11,14] This supports the possibility of medicinal and dietary manipulations as a feasible means of prevention and treatment of smaller enteroliths.

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