

Evaluation of Clays as Binding Agents for Reduction of Radionuclides in Milk

Binding Properties of Natural and Hydrogen Form Clays with Strontium and Essential Cations in Artificial Rumen and in Simulated Abomasal and Intestinal Fluids

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An *in vitro* screening device was developed to evaluate radionuclide binding materials for their possible use as feed additives in the diets of domestic ruminants to reduce the levels of radioactivity in milk and meat. Binding materials were tested first in an artificial rumen which was later converted to simulate the fluids of the abomasum and jejunum. A sodium and calcium montmorillonite clay and kaolin were tested in both their natural and hydrogen form.

None of these clays tested removed Sr^{2-} , Ca^{2-} , Mg^{2+} , K^+ , or Na^+ from the rumen juice or simulated digestive fluids to any considerable degree. On the contrary, bivalent cations originally present on the montmorillonites were removed from the clays into the media, increasing the levels of Sr^{2-} , Ca^{2-} , and Mg^{2+} . This was especially true of the calcium clay. This occurred to a lesser extent when these clays were converted to the hydrogen form.

Various naturally-occurring and synthetic substances have been shown to bind radionuclides in a variety of media. Some of these have been suggested as feed additives in diets of domestic ruminant animals so as to bind the nuclides in the gut to prevent their absorption and subsequent secretion into milk and meat. Testing of the many possible radionuclide-binding materials in cows or other ruminants would be both costly and time-consuming. An *in vitro* screening method to evaluate radionuclide-binding materials in the whole natural rumen juice and fluids simulating those of the abomasum and intestinal tract was developed by the authors. Further testing in large animals would be necessary only with materials which showed definite promise as radionuclide-binding agents in the gut.

The artificial rumen provides an *in vitro* procedure for testing binding materials in a natural medium, taken directly from a rumen fistulated cow, in the presence of viable rumen microflora. The effect of the microflora on the binding agent or the effect of the binding agent on the microflora can also be studied. A general discussion of artificial rumen techniques is given in Barnett and Reid (1961).

As an extension of the well-established artificial rumen procedures, a simulated bovine abomasal and intestinal fluid was developed. Simulated gastric fluid and simulated intestinal fluid have been used for drug release studies in the pharmaceutical field and are described in the U. S. Pharmacopoeia (1960). These fluids were modified to simulate the abomasal and jejunal conditions of the ruminant as closely as possible.

Only small amounts of the materials of interest are necessary for testing in this procedure. This procedure provides a means for sampling the contents of the various phases of the simulated digestive tract used in the investigations. The

results give indications as to where in the digestive tract binding or dissociation reactions may be taking place.

The cation exchange reactions of the various clays are well known. Clay materials have been proposed as possible binding agents of ^{90}Sr and ^{137}Cs in the gut of ruminants. Mraz and Patrick (1957) reported that both bentonite and vermiculite fed to rats greatly increased the fecal excretion of ^{134}Cs . Adams *et al.* (1965) stated that when 10% bentonite clay was added to sand plots ^{90}Sr and ^{137}Cs uptake by radishes was decreased by a factor of 6 and 3, respectively.

Wiklander (1964) stated that there is no single universal order of replacing power in clays. For alkali ions it is generally $\text{Li}^+ < \text{Na}^+ < \text{K}^+ < \text{Rb}^+ < \text{Cs}^+$; while for alkaline earth metal ions: $\text{Mg}^{2+} < \text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+}$. The position of the monovalent and divalent ions in a single combined series, has proved to vary considerably with the nature of the exchanger and the concentration of the ions in the solution. Gacinovic and Micic (1961) reported that the affinities of Ca^{2+} and Sr^{2+} on a montmorillonite type of clay are approximately equal.

Giesecking and Jenny (1936) reported the following order of replacing power of polyvalent cations on hydrogen form clay: $\text{Mg}^{2+} < \text{Ba}^{2+} < \text{Ca}^{2+} < \text{Sr}^{2+}$. In the case of hydrogen clay Ba^{2+} has shifted to a lower position.

The binding properties of two forms of three clays (in effect, six clays) with the cations of Sr, Ca, Mg, K, and Na in the artificial rumen and simulated abomasal and intestinal juices were investigated. Since the binding of stable and radioactive strontium is similar, and both would be bound simultaneously, only stable or total strontium was considered in this study. Calcium, magnesium, potassium, and sodium represent essential cationic minerals and their removal from these juices by binding materials is of interest.

The three clays, which were examined in their natural and hydrogen ion form were Belle Fourche bentonite (Volclay SPV, American Colloid Co.), Aberdeen bentonite (American Colloid Co.), and EPK kaolin (Edgar Plastic Kaolin Co.). Belle Fourche and Aberdeen bentonites represent a sodium and calcium form of montmorillonite clay, respectively, with a relatively high cation exchange capacity. EPK kaolin, on the other hand, represents a clay of relatively low cation exchange capacity.

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EQUIPMENT AND REAGENTS

Apparatus. Digestion flasks: A 300-ml. Erlenmeyer flask with a 2-hole rubber stopper was fitted with glass tubing extending almost to the bottom. An air reflux condenser was made out of a 10-ml. volumetric pipet shortened to leave a 2-inch stem at each end of the reservoir. This was fitted through the stopper so that one end extended as much as one-half inch into the flask.

Instruments. Beckman Zeromatic pH Meter equipped with a combination electrode and a Kontes Glass Chromaflex Column 500 × 25 mm. with the fritted disk replaced by platinum wire gauze were used.

Reagents. Strontium chloride solution containing 1.1 grams $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ per liter.

Simulated abomasal juice without pepsin. 8.0 grams of NaCl and 28 ml. of HCl made up to 1000 ml. with H_2O . Pepsin is added when used. (Sodium chloride will be omitted from this juice in future experiments.)

Pepsin. (Nutritional Biochemicals Corp.), 1–20,000 (6.6 × N.F.).

Bile. 20 grams of Bacto-oxgall to 200 ml. of H_2O (Difco Laboratories).

Simulated intestinal juice. To 150 ml. of 0.1*N* NaHCO_3 the following was added and mixed in blender: 18.75 grams pancreatin (U.S.P.) Nutritional Biochemicals Corp., 0.9375 gram trypsin, 4 × U.S.P. pancreatin (N.B.C.), 0.9375 gram erepsin (N.B.C.), sodium hydroxide, 5.0*N*.

PROCEDURE

Two quart-size thermos bottles of rumen juice were collected from a fistulated cow. Ten milliliters of strontium chloride solution were added to one thermos bottle at the time of collection. The entire contents of the bottle containing the added strontium was used. The balance of 1200 ml. of rumen juice was taken from the other bottle. This provided approximately 3.0 p.p.m. of strontium ion in the rumen juice.

A 1200-ml. portion of rumen juice was added to a 2000-ml. Erlenmeyer flask, and 120 ml. of 3% NaHCO_3 solution which had been saturated with CO_2 were added. The juice was saturated with CO_2 in a water bath set at 39.5° C. and the pH adjusted to 6.5 with saturated sodium carbonate solution.

Three-hundred-milliliter Erlenmeyer flasks containing 0.5 gram of clay and 0.75 gram of cellulose were inoculated with 100 ml. of the rumen juice preparation. Each flask was fitted with a stopper assembly consisting of the glass CO_2 intake tube and reflux condenser outlet previously described. The digestion flasks were incubated in a shaker bath at 39.5° C. for about 21 hours (overnight). A steady stream of CO_2 was passed through the contents of each flask throughout the digestion period.

Following the digestion period a 5-ml. aliquot was taken from each flask. The samples were centrifuged in plastic tubes at the maximum speed of a Servall table model angle centrifuge Type M (over 5000 r.p.m.). The supernatant fluid was collected for mineral analysis. Care was taken to avoid including any sediment in the samples to be analyzed.

The artificial rumen was then converted to a simulated abomasum. One-half gram of pepsin was added to 250 ml. of simulated abomasal juice. A 25-ml. portion of simulated abomasal juice was added to each flask and the pH adjusted to 3.0 with 5.0*N* HCl. The flasks were stoppered with cotton plugs and incubated in the shaker bath for 3 hours. Samples were again taken for analysis.

The simulated abomasum was then converted to simulate

the early jejunum. A 20-ml. portion of 0.1*N* NaHCO_3 was added to each flask followed by 20 ml. of bile. The flasks were incubated for 10 minutes. Ten milliliters of simulated intestinal juice, containing the enzymes, were added and the pH was adjusted to 6.0 with 5.0*N* NaOH. The flasks were returned to the shaker bath and incubated for 3 hours. Aliquots were taken for analysis.

The samples were analyzed for Sr^{2-} , Ca^{2-} , Mg^{2+} , K^+ , and Na^+ by means of a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer.

Hydrogen form clays were prepared by a procedure similar to one described by Lewis (1953). Essentially, a 2% clay suspension was prepared in 0.01*N* NH_4OH . A blender was used to disperse the clay. The clay suspension was passed slowly through a Dowex 50W-X8 ion exchange column fitted with platinum wire gauze in place of the fritted disk. In the case of the Belle Fourche and Aberdeen bentonite, 5 grams of clay were dispersed in 250 ml. of NH_4OH solution. In the case of EPK kaolin, 7 grams of clay were dispersed in 350 ml. of NH_4OH solution. The depth of the resin bed was usually about 14 inches. The pH of the effluents was read often to be sure that the resin had not become exhausted.

The dry matter content of the hydrogen form clay suspensions was determined. An equivalent volume of clay suspension equal to 0.5 gram of clay was pipetted into plastic centrifuge tubes and centrifuged at the maximum speed of a Servall table model angle centrifuge with a Type SP head (over 5000 r.p.m.). The supernatant fluid was decanted and the sediment resuspended in a small amount of water. The clay suspensions were transferred into the digestion flasks with a small amount of wash water.

Digestion flasks were prepared in duplicate for each treatment. The 3 clays were tested simultaneously under similar conditions in each flask. Three separate trials were run on the natural clays and 3 trials were run on the hydrogen form clays.

Each treatment was tested for significant difference from controls by Dunnett's method (1955, 1964).

RESULTS

The analytical results for soluble Sr^{2-} , Ca^{2-} , Mg^{2+} , K^+ , and Na^+ in the rumen juice and digestive fluids at the end of each phase are given in Tables I and II for the natural and hydrogen clays, respectively. For the controls the actual concentration in parts per million is given. For the clay treatments, percentages relative to the control values are given. The values in the tables represent the averages of 3 trials. "Soluble Cation Concentrations" includes only the cation content of the supernatant fluids which is actually free for binding by the clays. Minerals associated with the microflora or feed debris are not included.

The natural clays under the experimental conditions were not effective in the removal of Sr^{2-} during the rumen, abomasal, or intestinal phases. To the contrary, strontium and calcium originally present on the bentonites were removed from the clay into the fluids. The small observed increases in Mg^{2+} concentration were not statistically significant. Statistical significance is indicated in the tables.

The hydrogen form clays were not effective in the removal of Sr^{2+} to any large degree. Hydrogen form Belle Fourche bentonite removed a small portion of Sr^{2-} from the artificial rumen. The small observed reductions in concentration of the other cations were not statistically significant. However, these cations were, for the most part, returned to the fluid during the abomasal phase. Hydrogen form EPK

Table I. Soluble Cation Concentrations of Clay-Treated Fluids Following Digestion Periods

Natural Clays, Percentage Relative to Controls						
Digestive phase	Clay	Sr ²⁺	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺
Artificial rumen	Control (none)	2.76 p.p.m. 100%	107.0 p.p.m. 100%	76.00 p.p.m. 100%	1519 p.p.m. 100%	2939.6 p.p.m. 100%
	Belle Fourche bentonite	115.9	110.5	110.6	95.0	100.0
	Aberdeen bentonite	213.9 ^a	137.0 ^a	111.1	96.1	98.7
	EPK kaolin	99.5	99.9	106.8	97.6	97.6
Abomasal	Control (none)	2.67 p.p.m. 100%	152.5 p.p.m. 100%	68.7 p.p.m. 100%	1214.6 p.p.m. 100%	2935.4 p.p.m. 100%
	Belle Fourche bentonite	133.7 ^a	114.1	109.3	94.9	94.4
	Aberdeen bentonite	216.6 ^a	141.0 ^a	111.2	99.5	98.0
	EPK kaolin	98.2	101.5	97.1	96.3	95.1
Intestinal	Control (none)	1.6 p.p.m. 100%	94.8 p.p.m. 100%	38.9 p.p.m. 100%	723.7 p.p.m. 100%	4224.7 p.p.m. 100%
	Belle Fourche bentonite	133.8	109.3	106.4	100.4	99.6
	Aberdeen bentonite	219.8 ^a	129.2 ^a	101.1	97.7	98.1
	EPK kaolin	102.3	97.6	91.0	96.2	96.8
	Std. error	8.49	4.08	3.24	1.66	2.75

^a Significantly different from control at 5% level (P ≤ 0.05).

Table II. Soluble Cation Concentrations of Clay-Treated Fluids Following Digestion Periods

Hydrogen Form Clays, Percentage Relative to Controls						
Digestive phase	Clay	Sr ²⁺	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺
Artificial rumen	Control (none)	2.81 p.p.m. 100%	192.7 p.p.m. 100%	71.3 p.p.m. 100%	1180.2 p.p.m. 100%	2141.7 p.p.m. 100%
	Belle Fourche bentonite	87.9 ^a	88.6	89.3	92.2	92.4
	Aberdeen bentonite	132.0 ^a	107.7	101.0	94.6	96.3
	EPK kaolin	89.9 ^a	82.0	88.6 ^a	91.9	90.5
Abomasal	Control (none)	2.43 p.p.m. 100%	202.4 p.p.m. 100%	60.6 p.p.m. 100%	925 p.p.m. 100%	2197.9 p.p.m. 100%
	Belle Fourche bentonite	102.8	95.7	101.7	97.8	90.7
	Aberdeen bentonite	154.0 ^a	111.1	109.7	100.5	89.1
	EPK kaolin	100.0	100.7	92.3	94.2	82.8
Intestinal	Control (none)	1.76 p.p.m. 100%	144.5 p.p.m. 100%	43.2 p.p.m. 100%	751.3 p.p.m. 100%	4119.8 p.p.m. 100%
	Belle Fourche bentonite	96.5	95.2	93.5	93.6	92.1
	Aberdeen bentonite	139.4 ^a	107.1	101.9	98.3	93.9
	EPK kaolin	93.6	97.6	90.8	91.8	90.3
	Std. error	2.95	3.47	2.93	2.07	4.01

^a Significantly different from control at 5% level (P ≤ 0.05).

kaolin showed a small removal of Sr²⁺ and Mg²⁺ cations during the rumen phase. The small reductions in sodium concentrations observed in all three phases, in the case of all of the hydrogen form clays were not statistically significant.

DISCUSSION

Several possible factors are suggested in regard to the dissociation of the bivalent cations from clays in these media. The concentration of sodium in these media is far greater than that of strontium, calcium, or magnesium. Kelley and Cummins (1921) (as reported by Grim, 1953) found that the re-

placement of Ca²⁺ and Mg²⁺ by Na⁺ in the Yolo soil of California increased as the concentration of the Na⁺ in the solution increased. Grim (1953) stated that this is to be expected, since cation exchange is a stoichiometric reaction and the laws of mass action would hold. In general, therefore, increased concentration causes greater exchange by that cation. Kelley (1948) (as reported by Grim, 1953) had shown that with cation pairs of about similar replacing power and of the same valence such as K⁺ vs. NH₄⁺ or Ca²⁺ vs. Ba²⁺, dilution had relatively little effect on exchange, while with cations of different replacing power and different valence such as Na⁺ vs. Ca²⁺ or NH₄⁺ vs. Ca²⁺, dilution produced a marked effect

on exchange. Mishio and Tcho (1964) reported that the existence of NaNO_3 does not affect the decontamination of ^{137}Cs , in waste solutions, by an acid clay to a great extent. The decontamination of ^{89}Sr is, however, likely to be influenced by the existence of Na^+ and the decontamination ratio decreases appreciably.

The organic fraction and pH of these media may have played a role in the dissociation of bivalent cations from clays. Meyers (1937) (as reported by Ensminger and Giesecking, 1939) observed that organic colloids mixed with inorganic soil colloids showed a reduction in cation exchange capacity from the sum of the capacities of the two components. He also observed that the tendency of organic colloids to combine with inorganic soil colloids was greater in acid suspensions. Ensminger and Giesecking (1941) reported that proteins reduced the base exchange capacity of montmorillonite clay materially when the complexes were acidified.

Cho (1959) reported that in the case of bentonite the cation exchange capacity of the clay continually decreased with the addition of HCl. Prout (1958) reported that in soils consisting of 20% clay and white sand in which the dominant clay was kaolinite, the maximum absorption of Sr^{2+} occurred at pH 7. Strontium absorption was greatly inhibited by the hydrogen ion.

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