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

Binding Properties of Clays with ^{134}Cs in Artificial Rumen and in Simulated Abomasal and Intestinal Fluids, and Uptake of ^{134}Cs by Rumen Microflora

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The binding properties of ^{134}Cs by the natural and hydrogen form of sodium and calcium montmorillonite clays and kaolin were tested in an artificial rumen and simulated fluids of the abomasum and jejunum. Natural Belle Fourche bentonite (sodium montmorillonite) removed 82% of the radioactivity from the rumen juice. Natural Aberdeen bentonite (calcium montmorillonite) removed 69% of the ^{134}Cs from the rumen juice. In both instances there was dissociation of the clay cesium complex with some of the ^{134}Cs returning into the abomasal and and intestinal fluids. Hydrogen form Aberdeen bentonite removed 72% of the ^{134}Cs from the rumen juice. Following the intestinal phase, 57% of the radioactivity remained bound to the clay. The binding properties of Belle Fourche bentonite for ^{134}Cs decreased considerably when this clay was converted to the hydrogen form. Kaolin had no effect of practical significance. Results of an *in vitro* artificial rumen study to determine the extent of concentration of radiocesium by rumen microflora showed that less than 3% of the total radioactivity was associated with the microflora and other sediment.

The high affinity of certain clay materials for cesium is well known. Adams *et al.* (1965) stated that the addition of 10% bentonite clay to sand plots decreased the ^{137}Cs uptake by radishes by a factor of 3. Mraz and Patrick (1957a) observed that rats subcutaneously injected with ^{134}Cs and fed 10% bentonite experienced greatly increased fecal excretion of ^{134}Cs with concurrently decreased ratios of urinary to fecal ^{134}Cs as compared with others fed a basal diet. Mraz and Patrick (1957b) also observed that vermiculite and bentonite preferentially removed ^{134}Cs ions from solutions that also contained potassium, hydrogen, or sodium ions. Prout (1958) reported that in soils consisting of 20% clay and white sand in which the dominant clay was kaolinite, the maximum absorption of cesium occurred near pH 8. Mishio and Tcho (1964) reported that the presence of Na^+ does not affect the decontamination of ^{137}Cs from waste solutions by acid montmorillonite clay to as great an extent as with Sr^{2+} ion. Barth and Bruckner (1969) reported that the natural bentonites evaluated increased the content of Sr^{2+} and Ca^{2+} in the fluids of an artificial rumen and simulated abomasum and intestine. There was little effect on the levels of Mg^-, K^-, or Na^+. EPK kaolin had little effect on the levels of these cations.

This paper describes an *in vitro* study of the binding properties of ^{134}Cs by the natural and hydrogen forms of Belle Fourche bentonite (sodium montmorillonite, Volclay, S.P.V., American Colloid Co.), Aberdeen bentonite (calcium montmorillonite, American Colloid Co.), and EPK kaolin (Edgar Plastic Kaolin Co.).

An adjunct study to determine the uptake of ^{134}Cs by rumen microflora was also conducted. Certain bacteria are known to utilize and concentrate cesium (MacLeod and Snell, 1948, 1950). Certain moss and lichen vegetation are also known to accumulate ^{137}Cs (Svensson and Lidén, 1965a,b; Plummer and Helseth, 1965).

If the rumen bacteria or protozoa should preferentially utilize and concentrate cesium to a considerable extent, then a portion of the radiocesium present would not be available to be acted upon by binding agents until the microflora are digested in the abomasum or intestine. This could lower the efficiency of radionuclide binding agents administered to ruminants to reduce radiocesium in milk and meat.

PROCEDURE

The procedure is generally very similar to the artificial rumen and simulated abomasum and intestine described by Barth and Bruckner (1969). As a precautionary measure, to prevent the possible escape of radioactive fluid from the digestion flasks, a small amount of glass wool was inserted into the upper arm of each air reflux condenser. This served to break the bubbles that usually arose in the CO_2 outlet during these experiments.

Six-hundred milliliters of double strength basal medium were added to a 2000-ml. Ehrlenmeyer flask containing 12 grams of powdered cellulose. The flask was fitted with a gas intake stopper assembly with the reflux condenser made from a 50-ml. volumetric pipet. The flask was placed in the water bath and the contents were saturated with CO_2. The basal medium was double the strength of that described by Cheng *et al.* (1955).

Six-hundred milliliters of rumen juice were added to the flask containing the basal medium, and the contents were saturated with CO_2 for about 15 minutes. The pH was adjusted to 6.5 with saturated sodium carbonate solution.

The rumen juice was augmented with 0.27 μCi of ^{134}Cs and mixed thoroughly. The rumen juice was incubated for about 22 hours (overnight) with carbonation, and mixed by hand occasionally. The preliminary incubation period provided an opportunity for the ^{134}Cs to enter into any chemical or physiological reactions which might occur with the medium or microflora. At this point, samples of the juice were removed for the study of ^{134}Cs uptake by rumen microflora.

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Following the preliminary incubation period the rumen juice was again adjusted to pH 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. The flasks were stoppered and incubated in a shaker bath with carbonation. Following the rumen incubation period, the digestion flasks were converted to a simulated abomasum and intestine as described in detail in the previous paper by Barth and Bruckner, page 1340.

Following each incubation period a 5-ml. sample was taken from each flask and centrifuged in 15-ml. Corex thick-walled glass centrifuge tubes at the maximum speed of a Servall type M table model angle centrifuge (over 5000 r.p.m.). A 2-ml. sample of sediment-free supernatant liquid was pipetted directly into counting tubes for radioactivity analysis. Radioactive counting was done in a Nuclear Chicago Dual Channel Gamma Analyzer with automatic sample changer. Count controlled operation was used to obtain a maximum estimate of the standard deviation of the net counting rate of 3.6% (2:1 gross counts/background, 4000 counts/sample) as described by Taylor (1957).

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

Tests of significance of different treatments from controls were tested by means of Dunnett's method (1955, 1964).

To determine the extent of cesium uptake by rumen microflora, four rumen juice samples of 5 ml. each were removed from the flask for radioactivity analysis following the preliminary incubation period. Four more 5-ml. samples were taken from the incubation flask and centrifuged in 15-ml. Corex thick-walled glass centrifuge tubes at the maximum speed of a Servall table model type M Angle Centrifuge (over 5000 r.p.m.). A 2-ml. sample of sediment-free juice was pipetted directly into each of the counting tubes for radioactivity analysis.

The volume of sediment was determined as closely as possible by decanting and weighing the supernatant liquid. The specific gravity of the supernatant liquid was taken as unity. The volume of packed sediment was calculated by difference. Four separate trials were run.

RESULTS

The percentages of free ^{134}Cs remaining in the juices at the end of each digestive phase relative to the controls are shown in Table I. The percentages given for the natural clays represent the final averages for 4 separate trials with 2 flasks per treatment. The percentages given for the hydrogen-form clays represent averages of 3 trials.

Under the conditions of this experiment, natural Belle Fourche bentonite removed 82% of the radioactivity from the rumen juice. During the abomasal phase, there was some dissociation of the ^{134}Cs clay complex with some return of the cesium to the media.

Natural Aberdeen bentonite removed 69% of the ^{134}Cs from the rumen juice. Again there was dissociation of some ^{134}Cs in the abomasal and intestinal phase with consequent return of ^{134}Cs into the media.

Hydrogen form Aberdeen bentonite removed 72% of the ^{134}Cs from the rumen juice. However, following the intestinal phase, 57% of the radioactivity was bound to the clay.

Neither natural nor hydrogen-form EPK kaolin had an effect of practical significance on the levels of ^{134}Cs in the artificial rumen or abomasal and intestinal phases.

Table I. Percentage of Free ^{134}Cs Remaining in Clay-Treated Fluids Following Digestion Periods

Digestive Phase	Clay	Percentage Relative to Controls: Natural Form, %	Hydrogen Form, %
Artificial rumen	Control (none)	100	100
	Belle Fourche bentonite	17.7[a]	62.4[a]
	Aberdeen bentonite	31.3[a]	27.9[a]
	EPK kaolin	90.4[a]	85.3[a]
Abomasal	Control (none)	100	100
	Belle Fourche bentonite	32.0[a]	82.3[a]
	Aberdeen bentonite	50.3[a]	36.7[a]
	EPK kaolin	98.7	84.5[a]
Intestinal	Control (none)	100	100
	Belle Fourche bentonite	37.9[a]	84.3[a]
	Aberdeen bentonite	57.4[a]	42.7[a]
	EPK kaolin	96.7	89.8
	Std. error	3.75	3.80

[a] Significantly different from control at 5% level ($p \leq 0.05$).

Uptake of ^{134}Cs by Rumen Microflora. Following the 22-hour preliminary incubation period, an average of 97.37% of the radioactivity was found in the sediment-free portion of the juice. Following centrifugation, the sediment, consisting mainly of microflora, feed debris, and cellulose, made up an average of 13.62% of the juice by volume. Since the rumen juice was diluted by one half with basal medium mixture, the volume of sediment in the liquid portion of the natural rumen contents would probably be higher.

The distribution of the ^{134}Cs radioactivity in an artificial rumen suspension under the conditions of this experiment, following a 22-hour digestion period, is shown below:

Counts per minute per 1 ml. of total whole juice:	98.3
Counts per minute per 1 ml. of supernatant liquid:	109.6
Counts per minute per 1 ml. of sediment:	30.0

Less than 3% of the total radioactivity was associated with the microflora and other sediment. Although the radioactivity per milliliter in the supernatant liquid was 3.6 times greater than the radioactivity per milliliter of sediment, the supernatant comprised an average of 86% of the whole juice.

DISCUSSION

Natural Belle Fourche bentonite and hydrogen form Aberdeen bentonite bound ^{134}Cs in these juices to a considerable degree with natural Belle Fourche bentonite being superior to the other clays tested. This is in contrast to the results obtained when the binding properties of strontium were evaluated for these clays, using this same procedure (Barth and Bruckner, page 1340).

In all cases, binding of ^{134}Cs by these clays was most efficient in the artificial rumen with dissociation of ^{134}Cs from the clay back into the juices in the abomasal and intestinal phases.

During the preparation of the simulated abomasal fluid,

the acidity was adjusted to 3. Cho (1959) reported that in the case of bentonite, the cation exchange capacity of the clay continually decreased with the addition of HCl. Although the fluid was later readjusted to pH 6 in the intestinal phase, the clays did not regain the binding properties which were shown in the artificial rumen during the incubation time allowed. On the contrary, dissociation of the complex resulting in return of the ^{134}Cs to the juices increased during the intestinal incubation period.

Ensminger and Gieseking (1941) reported that proteins did not reduce the base exchange capacity of clays in an alkaline medium but did reduce the base exchange capacity of montmorillonite clay (Wyoming bentonite) materially when the complexes were acidified. Increase in hydrogen ion concentration caused protein to act more like a cation. Meyers (1937) (as reported by Ensminger and Gieseking, 1939) observed that organic colloids mixed with inorganic soil colloids showed a reduction in cation exchange capacity; and that the tendency of organic colloids to combine with inorganic soil colloids was greater in acid suspensions. Ensminger and Gieseking (1939) reported that in Wyoming bentonite proteins were more completely absorbed in suspensions with high hydrogen-ion concentrations.

The trials examining the natural and hydrogen form clays were run at different periods involving different controls. However, a considerable reduction in ^{134}Cs binding by hydrogen form Belle Fourche bentonite as compared to the natural form is observed. When natural Belle Fourche bentonite was converted to the hydrogen form, exchangeable sodium was replaced by hydrogen. The binding of ^{134}Cs may have been reduced because hydrogen is probably more difficult to replace than sodium.

Uptake of ^{134}Cs by Rumen Microflora. Under the conditions of the artificial rumen, the rumen microflora did not concentrate ^{134}Cs. The ^{134}Cs associated with the rumen microflora and other sediment is negligible. The majority of the ^{134}Cs radioactivity was in a free soluble form and available to be acted upon by radionuclide binding agents administered to ruminants to reduce the radiocesium in milk and meat. This applies only to the radiocesium found in the juice, and not that which is an integral part of undigested plant materials in the rumen.

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