Evaluation of Certain Properties of Radiocerium as an Indigestible Marker

J. E. Huston and W. C. Ellis

Five experiments were conducted to study the affinity of radiocerium for feedstuff and digesta particles under in vitro conditions simulating the intraruminal environment. The collective results indicated radiocerium was rapidly adsorbed on and remained tenaciously bound to digesta particles, and might therefore be expected to move through the rumen in association with digesta particles resulting in a low variation in its fecal concentration. When fed, mixed with the ration, radiocerium was excreted with less variation than was either polyethylene glycol or chromic oxide.

The rare earth elements, in addition to being essentially indigestible by mammals (Ellis, 1968; Kyker, 1961), possess strong binding properties for particulate matter which, if maintained throughout the gastrointestinal tract, might make them very desirable indigestible markers. Another report from this laboratory indicated a relatively low variation in the fecal concentration of the rare earth element dysprosium when it was adsorbed onto dietary particles fed to sheep (Ellis, 1968). Experimental verification that adsorption of dysprosium onto feedstuff residues was maintained throughout the gastrointestinal tract was not provided because of the low concentrations at which adsorption occurs (10^{-7} to $10^{-11}M$) (Kyker, 1961; Schweitzer and Jackson, 1952) and the limited sensitivity of the radioactivation method employed (calculated to be equivalent to $10^{-4}M$ in the rumen). The present report involves results obtained with a radioisotope of cerium which, due to the sensitivity of its radioassay, permitted studies at concentrations below $10^{-11}M$—concentrations at which the rare earths exhibit strong adsorptive properties termed "radiocolloidal behavior" (Schweitzer and Jackson, 1952). A preliminary report of these results has been presented (Huston and Ellis, 1965).

MATERIALS AND METHODS

The radiocerium used was a processed, carrier-free, chloride salt of the isotopes ^{144}Ce–^{144}Pr obtained from Oak Ridge National Laboratory, Oak Ridge, Tenn. Stock solutions of approximately $10^{-11}M$ were prepared in 0.1N hydrochloric acid for the in vitro experiments and $10^{-10}M$ in 0.1N hydrochloric acid for the in vivo experiment. Adsorption of the radionuclide onto glassware at this low concentration was effectively minimized by coating all involved glassware with a water-repellent silicone film of Dessicote (Beckman Instruments, Inc., Fullerton, Calif.) (Schweitzer and Jackson, 1952).

Radioassay was performed with a scaler connected to either a 5.08 × 5.08 cm. well-type sodium iodide (TI) scintillation detector or a 5.08-cm. diameter thin window, gas flow detector operated in the Geiger region. The materials were variously prepared for counting as described for each individual experiment.

Department of Animal Science, Texas A&M University, College Station, Tex. 77843

PROCEDURE

General Approach. Five in vitro experiments were conducted to study factors affecting the adsorption of radiocerium onto feedstuff particles (experiment I), its distribution onto different size particles of rumen fluid (experiments II and III) and rumen contents (experiment IV), and its redistribution following the fermentation of feedstuff particles upon which the radionuclide had been adsorbed (experiment V). The criterion for adsorption by the radiocerium was either retention on a filter (experiment I) or sedimentation (experiments II, III, IV, and V) with the demonstrated lack of sedimentation under similar experimental conditions but without adsorbent. A sixth experiment was conducted to test the in vivo implications of the in vitro experiments.

More specific details of the procedure are either given below or in connection with the tabular results.

Experiment I. Aliquots of alfalfa hay, ground to pass a screen having 1.27-cm. square openings, were added to centrifuge tubes containing 50 ml. of one of the media indicated in Table I.

Experiments II and III. Rumen contents were obtained from a fistulated steer fed sorghum hay ground to pass a 2.54-cm. screen. Duplicate samples of digesta (approximately 20 grams) were placed in a 50-ml. centrifuge tube, and 10^{-11} mmole of radiocerium (2.2 × 10 d.p.m.) was added and mixed with a glass rod. The tubes were incubated at 39° C. for the designated time intervals and then removed and fractions centrifuged as indicated in Table II. The centrifuged residue from each successive fraction was dried and weighed to permit calculations of specific activity (Table III, radioactivity per unit weight). Radioassay was performed on aliquots of the supernatant fluid.

Experiment IV. One hundred milliliters of rumen contents was added to each of 15 250-ml. Erlenmeyer flasks, 1.5 × 10^{-11} mmole of radiocerium (approximately 3.3 × 10^5 d.p.m.) was added, and the flask was incubated at 39° C. Triplicate flasks were removed after 0, 2, 4, 8, and 16 hours, and the particulate matter was separated into three different fractions by sequential passage through wire sieves of 1.27-, 0.68-, and 0.34-cm. square openings. The residue retained on the largest (uppermost) sieve was resuspended in the filtrate of all three other sieves and refiltered with the filtrate washing the residues retained by

Table I. Influence of pH, Electrolyte Concentration, and Incubation Time on the Adsorption of Radiocerium onto Alfalfa Hay Particles, Experiment I

Media	% of Total Radioactivity Retained after: Initial Filtrate, 1-hour incubation	Initial Filtrate, 24-hour incubation	pH 5 Wash, 1-hour incubation	pH 5 Wash, 24-hour incubation	pH 2 Wash, 1-hour incubation	pH 2 Wash, 24-hour incubation
pH 3						
HCl	83.3	96.9	77.0	96.2	74.8	91.7
Artificial saliva	60.0	63.6	55.0	56.6	53.5	52.6
pH 6						
HCl	60.6	96.6	55.9	96.2	50.4	92.2
Artificial saliva	47.2	77.0	36.2	63.2	12.2	47.4

Table II. Affinity of Radiocerium for Sedimentable Particulate Matter of Rumen Contents, Experiments II and III

Residual Fraction Min.	Residual Fraction × G	Hours Incubation with Rumen Contents: Expt. III, 0	Expt. III, 3	Expt. III, 8	Expt. II, 18
		Radioactivity precipitated, % of added[a]			
10	110	...	...	50.5	80.7
10	1200	73.2[b]	90.4	39.4	12.9
30	2000	15.4	5.2	6.7	1.1
30	3000	5.2	0.8	1.8	0.5
Accumulative total, %		93.8	96.4	98.4	95.2

[a] Calculated as $100 - \frac{\text{(ml. supernatant fluid)(c.p.m./ml.)}}{\text{(c.p.m. added to sample)}} \times 100$

[b] Each value a mean of duplicate samples.

Table III. Specific Activity of Fractions of Rumen Contents Subsequent to Incubation with Radiocerium, Experiments II and III

Sedimented Fraction Min.	Sedimented Fraction × G	Hours Incubated with Rumen Contents: Expt. III, 0	Expt. III, 3	Expt. III, 8	Expt. II, 18
		D.p.m. × 10^5/gram d.m. precipitated			
10	110	...	...	1.0	...
10	1200	1.5	1.7	16.0	20.4
30	2000	59.0	11.0	7.0	2.3
30	3000	58.0	80.0	26.0	17.7

the successively smaller and lower sieves. This washing process was repeated with water.

The combined filtrates and washing were further fractionated by sedimentation (Table IV). Aliquots of the filtrate and supernatant fluids were radioassayed using the crystal scintillation detector. The residues and sediments were removed, dried, weighed, and ashed for subsequent radioassay using the crystal scintillation detector. Counting efficiency was 64.7% for radioassay of the ashed samples as compared with only 2.6% for the liquid samples reflecting the low energies of the gamma radiation involved.

Experiment V. A fraction of alfalfa hay (18 grams) which passed through a 0.16-cm. sieve was soaked for 18 hours in 60 ml. of $10^{-11}M$ $^{144}CeCl_2$ (13.32×10^6 d.p.m.) and subsequently dried. Each of 15 incubation tubes was charged with 1 gram each of: alfalfa hay particles which were retained by a 1.27-cm. sieve; alfalfa hay particles which passed through a 0.16-cm. sieve; and the radiocerium-labeled alfalfa hay particles. Forty milliliters of rumen fluid was added to 12 tubes which were then incubated at 39° C., with triplicate tubes being removed after 1, 2, 4, and 16 hours and frozen. Forty milliliters of artificial saliva (McDougall, 1948) was added to the remaining three tubes which were retained as a zero time blank and frozen.

The samples were later thawed and fractionated by sieves and sedimentation according to particulate size as indicated in Table V. Radioassay was as described for experiment IV.

Experiment VI. Approximately 200 μc. of radiocerium was adsorbed by soaking and drying onto 1 kg. of alfalfa, which had been ground through a 1.27-cm. screen. This radiocerium-labeled hay, 100 grams of Cr_2O_3, and 40 grams of PEG (the Cr_2O_3 and PEG premixed with 1 kg. of ground hay) were mixed in a vertical auger mixer with 45.4 kg. of similarly ground alfalfa hay.

Three mature wethers, previously adjusted to the experimental ration and environment, were each fed 600 grams of the ration twice daily (7:00 A.M. and 6:00 P.M.) during a 9-day preliminary period and an 80-hour fecal collection period. Feces were collected at 4-hour intervals and weighed; a 10-gram aliquot was taken for PEG determination (Smith, 1959) and the remainder dried and weighed. Thirty grams of the dried unground feces was placed in a polyethylene cup, which was then positioned in a reproducible manner over the thin-windowed, gas flow detector for radiocerium assay. Counting efficiency was 0.88% by this procedure. An aliquot of the dried feces was ground in a high speed blender for subsequent analysis for chromic oxide (Kimura and Miller, 1957).

RESULTS AND DISCUSSION

Table I contains results of the experiment designed to study the influence of pH incubation time and electrolyte concentration on adsorption of radiocerium onto alfalfa hay particles. Inferences which can be made from this experiment are limited owing to the apparent penetration of the 180- to 220-micron filter by matter "solubilized" from the alfalfa particles. This was most noticeable for the artificial saliva medium filtrate, which was extremely opaque and turbid in contrast to that for the hydrochloric acid medium or the artificial saliva blank similarly treated except for added forage particles. Presumably, such nonfilterable material solubilized by the artificial saliva would have radiocerium adsorbed to it, as is suggested by comparing the results in Table I for the hydrochloric acid

Table IV. Distribution and Specific Activities[a] of Radiocerium and Dry Matter on Different Fractions of Rumen Contents after Different Incubation Periods, Experiment IV

	Fraction	Measurement	Incubation Time, Hour (T) 0	2	4	8	16	Regression Coefficient, S.A./T
1	1.27-cm. sieve	Sp. activity[a] (s.a.)	5.34[b]	6.77	7.41	7.81	10.40	0.28[c]
		Relative s.a.[d]	0.33	0.39	0.38	0.39	0.42	
2	0.68-cm. sieve	Sp. activity[a] (s.a.)	8.74	9.70	11.25	12.85	14.58	0.34[c]
		Relative s.a.[d]	0.54	0.56	0.57	0.65	0.59	
3	0.34-cm. sieve	Sp. activity[a] (s.a.)	16.29	17.42	19.67	19.88	24.84	0.51[c]
		Relative s.a.[d]	1.00	1.00	1.00	1.00	1.00	
4	× 250 G sediment	Sp. activity[a] (s.a.)	33.57	38.96	43.20	46.02	60.41	1.58[c]
		Relative s.a.[d]	2.06	2.24	2.20	2.31	2.43	
5	× 1200 G sediment	Sp. activity[a] (s.a.)	173.9	150.0	178.8	169.8	146.8	−1.21[e]
		Relative s.a.[d]	10.7	8.6	9.1	8.5	5.9	
								D.P.M., % of Total/T
1	1.27-cm. sieve	D.p.m., % total[f]	15.0[b]	18.7	17.6	17.0	23.9	0.46[e]
		D.m., % total[g]	51.2[b]	52.4	50.7	47.8	56.7	
2	0.68-cm. sieve	D.p.m., % total	7.3	8.7	7.4	10.7	6.8	−0.03
		D.m., % total	15.1	16.7	14.2	18.3	11.3	
3	0.34-cm. sieve	D.p.m., % total	13.0	12.1	15.6	13.4	10.0	−0.05
		D.m., % total	14.7	13.2	17.2	14.7	12.7	
4	× 250 G sediment	D.p.m., % total	31.5	33.3	33.0	36.9	42.5	0.69[e]
		D.m., % total	17.6	16.3	16.7	17.7	17.5	
5	× 1200 G sediment	D.p.m., % total	12.7	10.6	10.9	10.0	10.0	−0.13[e]
		D.m., % total	1.3	1.4	1.2	1.4	1.7	
6	× 1200 G supernatant	D.p.m., % total	20.5	16.6	15.4	11.9	4.7	−0.94[e]

[a] D.p.m. × 10^3 per gram of d.m.
[b] Each value a mean of three samples.
[c] $P = <0.01$.
[d] Relative specific activity; specific activity of indicated fraction divided by specific activity of fraction 3 for the corresponding incubation time.
[e] $P = <0.05$.
[f] Expressed as % of total radioactivity recovered.
[g] Expressed as % of summated dry matter in fractions 1 through 5; fraction 6, dry matter content considered negligible.

Table V. Redistribution of Radiocerium Previously Adsorbed onto 0.16-Cm. Feed Fractions during Incubation with Rumen Microorganisms, Experiment V

	Fraction	Measurement	Incubation Time, Hours 0	1	2	4	16
1	>0.32 cm.	C.p.m., % total	0.5[a]	3.2	1.9	1.1	3.3
		D.m., % total	8.0[a]	11.3	12.4	12.0	12.2
		C.p.m./g. d.m.	0.4[a]	1.8	1.1	0.6	1.7
2	<0.32 cm. >0.16 cm.	C.p.m., % total	3.9	7.7	9.3	8.2	10.2
		D.m., % total	20.4	19.0	22.5	25.1	24.7
		C.p.m./g. d.m.	1.5	2.7	3.0	2.3	2.7
3	<0.16 cm. sediment 1 × G	C.p.m., % total	94.3	80.8	79.3	86.9	68.0
		D.m., % total	68.9	59.7	56.9	58.4	56.7
		C.p.m./g. d.m.	10.7	9.2	10.1	10.1	7.9
4	sediment 1200 × G	C.p.m., % total	0.8	6.7	6.6	2.2	9.3
		D.m., % total	2.8	10.0	8.2	4.2	6.5
		C.p.m./g. d.m.	2.3	4.4	5.8	3.5	9.4
5	1200 × G supernatant	C.p.m., % total	0.4	1.6	2.9	1.6	8.5

[a] Each value a mean of three samples.

with the artificial saliva medium. Thus, the quantity of radiocerium retained by the filter must be considered a minimal measure of the radionuclide's affinity for alfalfa particles.

If the proportion of particulate matter passing through the filter was not increased with increasing incubation time, the degree of radiocerium adsorption onto particulate matter appeared to have increased with increasing contact time (incubation time) between 1 and 24 hours. This was true for each medium pH combination, although the effect was least for the pH 3 artificial saliva. These results suggest that a high degree of adsorption by radiocerium onto particulate matter (greater than 90%) required at least 1 and possibly as many as 24 hours. This suggestion was further supported by the relatively small amount of radiocerium removed by either the pH 5 or pH 2 buffer wash of the filterable residues after 24 hours as compared with after 1 hour of contact time in each medium. The pH of the initial medium did not appear to affect markedly the degree of initial adsorption. Neither did the pH of the wash buffers appear to affect markedly the degree of removal after 24 hours' contact time. These latter results suggest a tenacious binding of radiocerium onto the alfalfa particles.

Collectively, within the limitations imposed by the filtration techniques employed, the results of experiment I suggested that pH and electrolyte concentration had little influence upon the rate, degree, or tenacity with which radiocerium was adsorbed onto feed particles. Contact time of the hay with radiocerium in the medium appeared to have been the principal variable influencing these adsorptive properties.

Experiments II and III utilized centrifugal sedimentation as a criterion of radiocerium adsorption onto particulate matter. The validity of these criterion was indicated by the failure to sediment detectable amounts of radiocerium when it was added at concentrations below $10^{-11}M$ to particulate free artificial saliva (Table II). This is in contrast to the sedimentation of the rare earth dysprosium from artificial saliva at concentrations of approximately $10^{-5}M$ (Ellis, 1968). The results in Table II thus confirm a high affinity of ionic radiocerium at concentrations lower than $10^{-11}M$ for the sedimentable matter of rumen contents.

The magnitude of radiocerium sedimented from rumen contents was surprisingly large in view of the relatively low centrifugal forces employed, which would not have sedimented many bacterial species, and the possibility of soluble complex formation by cerium with colloidal macromolecules such as protein or soluble complexing molecules. The latter possibility is exemplified by the investigation of Johnson and Kyker (1961). These investigators added radiocerium (molarity not stated) to nutrient broth media inoculated with different bacteria. The amount of radiocerium precipitated by 18,000 $\times$ G (following variable growth periods) was related to the magnitude of bacterial growth. Such results suggested to Johnson and Kyker that citrate (added to media at $10^{-3}M$) or protein complexing prevented sedimentation of the radiocerium not removed by bacterial "uptake." Compared with the broth media employed by Johnson and Kyker, rumen contents would be expected to contain similar if not higher concentrations of colloidal proteins and soluble molecules capable of complexing with radiocerium and opposing its sedimentation.

Johnson and Kyker (1961) interpreted their results as indicative of an active metabolic uptake of cerium by bacteria. Only limited active uptake of cerium by bacteria might be expected to occur during the short incubation periods with rumen contents employed in the present experiments. However, a passive, adsorptive "uptake" could occur much more rapidly. This would be reflected by a specific activity (radioactivity per unit of weight of dry matter) inversely related to particle size (or directly related to surface area per unit of weight). Such specific activity data from experiments II and III are summarized in Table III. Possible interpretations of these results involve the basic assumption that a reduction in particle size was the only factor determining the dry matter precipitated by increasing centrifugal force.

There was, with the exception of the zero time 3000 $\times$ G fraction, an increase in specific activity in those fractions precipitated by increasing force from the zero- and 3-hour sample of rumen contents. This would suggest that the initial affinity of radiocerium for particulate matter of rumen contents is an adsorptive process related to the surface area of the particles. The apparent exception to this was the zero-hour flask, in contrast to the 3-hour flask, which might be attributed to an inadequate dispersion of radiocerium in the zero flask coupled with a relatively slow adsorption by radiocerium (as implied by the results from experiment I).

The specific activities of the 3000 and 2000 $\times$ G fractions, with the exception of the 3-hour, 3000 $\times$ G fraction, decreased with increasing incubation time. The specific activities of the 1200 $\times$ G fractions increased with increasing incubation time. Such changes might reflect an initial adsorption by radiocerium onto free microorganisms yielding high specific activity bacterial particles which subsequently became attached to larger feed particles being actively fermented and were precipitated therewith. A relatively slow initial adsorption could have occurred and could account for the increase in specific activity of the 3-hour, 3000 $\times$ G fraction.

The fractions of rumen fluid involved in experiments II and III represented only a limited array of particle sizes relative to that usually found in rumen contents. Interpretations as to the adsorptive fate of radiocerium in rumen contents are therefore limited. Experiment IV was conducted to study the distribution by radiocerium throughout a wider array of particle sizes. Fractionation of the larger particles of rumen contents (>1 $\times$ G) on the basis of size was difficult due to the abundance, and intermeshing effects, of heterogeneously sized particles. This resulted in the retention of relatively small particles by larger particles on relatively large size sieves. However, the procedure developed was sufficiently reproducible to afford a measure of the distribution of radiocerium and dry matter within a wide array of digesta particle sizes. Results from this experiment are summarized in Table IV.

Immediately after the addition of radiocerium (zero hour), the radionuclide was distributed such that its specific activity (radioactivity per unit dry weight of fraction) among the different fractions was inversely related to the fractions' particle size (Table IV). Although the surface area of the different fractions' particles was not determined, one would expect a similar order of difference in their surface area per unit weight as was suggested by the differences in specific activity. The authors therefore concluded that the affinity of radiocerium for the particulate matter of rumen contents was most likely an adsorptive effect related to the particle's surface area.

The specific activity of fractions 1 through 4 significantly increased ($P = <0.01$) and that of fraction 5 significantly decreased ($P = <0.05$) with increasing incubation time. This increase in specific activity by fractions 1 through 4 could have been the consequence of dry matter digestion of these fractions without alteration in their particle size (and hence retention within the same fraction as previously noted). However, a comparison of the distribution of radioactivity and dry matter by fraction and with incubation time does not support dry matter digestibility as the sole factor contributing to the changes in specific activity (Table IV).

There was no significant change in the distribution of dry matter among the different fractions with increasing incubation time. Thus there was no significant differential rate of dry matter disappearance from the different fractions. However, there were significant differences in

the percentage distribution of radioactivity and its direction of change in the different fractions with increasing incubation time. Radioactivity in the smaller particle fractions (5 and 6) significantly decreased during incubation, a decrease which was essentially accounted for by increases in the percentage of radiocerium recovered in fractions 1 and 4. Thus there appears to have been a considerable transfer of radiocerium from the two smaller particulate fractions (5 and 6) to a larger (fraction 4) and a much more coarse fraction (fraction 1) without net changes in dry matter distribution between any of the fractions.

These results suggest that, when added in solution to rumen contents, appreciable amounts of ionic radiocerium were adsorbed onto microorganisms (as a consequence of their mass and large surface area) which subsequently migrated to and became adherent to larger particles within fractions 1 and 4. Such a migration of high specific activity particles would result in an increase in specific activity of these fractions (1 and 4) relative to that for fractions in which the percentage of radioactivity remained relatively constant (fractions 2 and 3). Calculations of relative specific activity of each fraction relative to fraction 3 (Table IV) confirmed this expectation.

The progressive and relative large decline in the distribution of radiocerium (as measured both by percentage distribution and relative specific activity in Table IV) within the smaller particulate fractions (5 and 6) with increasing incubation time suggests a tenacious initial adsorption with limited subsequent exchange of radiocerium. Otherwise, had subsequent exchange occurred, a considerable transfer to the smaller fractions would have occurred (as a result of their greater surface area) in opposition to the observed result.

The question of redistribution by radiocerium was further investigated in experiment V in which radiocerium was prior adsorbed onto particles of defined size and subsequently incubated together with larger and smaller particles in rumen fluid (Table VI).

Had no redistribution of radiocerium occurred, the major portion of the radiocerium should have been recovered in the fraction onto which the radiocerium was initially adsorbed. Some radiocerium would, however, be transferred to fractions of smaller particle size by fermentative reduction in size of the particles onto which the radiocerium was initially adsorbed and by solution of dry matter onto which the radiocerium was initially adsorbed by the solvent properties of rumen fluid (Hendrick and Martin, 1963). An attempt was made to provide a correction for the latter by using artificial saliva as the solvent in the zero-hour incubation flask. However, a comparison of the distribution of dry matter and radiocerium in the artificial saliva (zero-hour) and the 1-, 2-, and 4-hour flask indicated that the particles' dry matter was much more soluble in rumen fluid than artificial saliva.

The results for the 4-hour incubation represented a departure from the trend suggested for the various fractions by considering the results of the 1-, 2-, and 16-hour data. This deviation was consistent for each individual of the triplicate samples and was likely the result of some unrecognized difference in the manner in which these samples were treated and/or fractionated relative to the 1-, 2-, and 16-hour samples. Therefore, only the latter samples were considered as comparatively valid. Analysis of variance associated with these results indicated: There was no statistical significance in any measurement due to incubation time in fractions 1 and 2; there was a significant decrease in the distribution of radiocerium and its specific activity in fraction 3 (which initially contained the radiocerium) with increasing incubation time, without a significant decrease in the distribution of dry matter therein; there was a significant increase in the distribution of radiocerium and its specific activity and a reduction in the distribution of dry matter with increasing incubation time in fraction 4; and there was a significant increase in the distribution of radiocerium into fraction 5 with increasing incubation time.

The direction of transfer of radiocerium with increasing incubation time when the radionuclide was prior adsorbed onto feedstuff particles (experiment V) was, in general, opposite to that observed in the previous experiment, where the ionic radionuclide was added and initially adsorbed in large amounts onto particles equivalent to those in fractions 4 and 5 of the present experiment. The increase in radiocerium within fractions 4 and 5 of the present experiment, with increasing incubation time, would thus appear to be radiocerium in continued association with the dry matter upon which it was originally adsorbed. Otherwise, had extensive transfer of radiocerium to other components of the 1200 × G sediment and supernatant occurred, a decrease in the distribution and specific activity within these fractions would have been expected to occur as observed in experiments II and III (Tables II and III) and experiment IV (Table IV), where these fractions were more directly labeled with radiocerium. Similarly, an increase in the distribution and specific activity of radiocerium within the more coarse fractions in experiment V would have been expected had extensive transfer of the previously adsorbed radiocerium occurred and of necessity involved adsorption onto motile microorganisms to a high specific activity.

The collective and comparative results of these in vitro experiments were interpreted to support the thesis that radiocerium previously adsorbed onto feedstuff particulate matter remains adsorbed on the undigested matter and that which was previously adsorbed onto matter subsequently digested would be readsorbed onto other digesta solids. Such properties would be advantageous in reducing diurnal variation in fecal marker concentration to the extent that such variations are the result of a dis-

Table VI. Coefficients of Variation for Concentration of Radiocerium, Chromic Oxide, and Polyethylene Glycol in 20 Fecal Collections from Each of Three Sheep, Experiment IV

Markers	Coefficients of Variation		
	Sheep	Collections	Duplicates
Radiocerium	12.4	6.4	2.7[a]
Chromic oxide	50.2	15.3	5.8
Polyethylene glycol	67.5	26.0	6.0

[a] There was no duplication of radiocerium activity counting due to limited feces. However, a pilot counting procedure gave an estimate of counting variation from which the coefficient of variation between duplicates was estimated.

sociated flow by marker and indigestible dry matter (Corbett *et al.*, 1959). This application was tested in experiment VI in which variations in the fecal concentration of radiocerium were compared with those for chromic oxide and polyethylene glycol.

The individual data for each 4-hour collection period are presented graphically in Figure 1. Inspection of these data does not suggest any discernible pattern of excretion by any marker. The lack of such an excretion pattern might be attributable to the relatively finely ground and homogeneous nature of the diet and the uniformity with which each marker was admixed.

The variation in each marker's fecal concentration attributable to analytical determination (duplicates), collection periods, and sheep was calculated and is expressed as coefficients of variation in Table VI. The variation in fecal concentration of radiocerium between the 4-hour collection periods was approximately twice the variation associated with its determination. Its variation between sheep was approximately twice its variation between collection periods. The variation in fecal concentration of chromic oxide between collection periods was approximately three times the variation associated with its determination and its variation between sheep approximately three times its variation between collection periods. Variation in fecal PEG concentrations was even larger than that for chromic oxide. Thus, there was considerably less variation between collection periods and sheep in the fecal concentration of radiocerium than for either chromic oxide or PEG. The results of the in vitro experiments suggesting a tenacious adsorption onto feedstuff dry matter by radiocerium, with the suggestion that variation in fecal marker concentration is in part a consequence of a dissociated flow by marker and dry matter from the rumen, suggest that the lower variation in fecal radiocerium concentration is a consequence of an associated flow by undigested matter and marker through and from the rumen.

An associated flow by radioyttrium and dietary residues through the rat's gastrointestinal tract has been suggested by Marcus and Lengemann (1962). These investigators used sedimentation of radioyttrium as the criterion for binding of the radionuclide onto digesta particulate matter. However, they did not demonstrate that such sedimentation of radioyttrium was the result of adsorption. Whether or not adsorption of rare earth cations onto digesta residues (in the context and by the criteria employed in the present experiments) is necessary to minimize diurnal variation in such cations' concentration in the fecal dry matter is not established. For example, a similar relatively low variation in fecal concentration of dysprosium was found when the concentration of this rare earth was sufficiently large ($10^{-5}M$) in rumen fluid to result in insoluble hydroxide formation—a condition which was in fact demonstrated to occur in vitro in artificial saliva when ionic dysprosium was added to a similar molar concentration (Ellis, 1968).

The variation in fecal radiocerium concentration between collection periods reported in this experiment is comparable

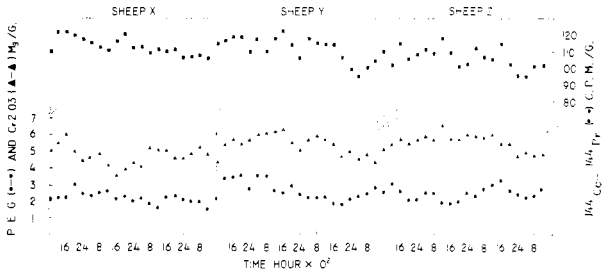

Figure 1. Variation in different markers' concentration in feces

with that previously reported for dysprosium when it was admixed with ground hay and determined by radioactivation analysis (Ellis, 1968). Coefficients of variations between daily collection periods for individual sheep of 8.9, 6.7, and 7.2 and within 4- to 6-hour collection periods of 3.4 and 8.7 were reported. The coefficients of variation associated with the radioactivation determination of dysprosium were 2.9 and those for its variation in the daily feed intake were 3.4. Thus, the period variation in fecal concentration of dysprosium was also approximately twice the variation associated with its determination. The variation between animals, although not calculated, was obviously low and of a similar order as found for radiocerium in the present experiment.

The relatively low variation in fecal radiocerium concentration reported here is also in accordance with the observations of Garner *et al.* (1960).

The magnitude of the differences between sheep in variation in fecal chromic oxide and PEG as compared with fecal radiocerium concentrations is surprisingly large. These differences suggest marked variation between individual sheep in the extent of dissociated flow of dry matter and chromic oxide or PEG. Variations in fecal concentrations of radiocerium between sheep were, in contrast to those for chromic oxide and PEG, markedly lower.

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