Dysprosium as an Indigestible Marker and Its Determination by Radioactivation Analysis

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A sensitive method is described for the instrumental radioactivation analysis of dysprosium in forages, rumen contents, and feces. The indigestibility of dysprosium was demonstrated. The variation in fecal dysprosium concentration between and within daily samples of feces was between two and three times its variation in concentration on the ingested forage upon which it had been adsorbed. Possible reasons for this relatively low diurnal variation are discussed.

The rare earth elements possess a number of properties which suggest their advantageous use as indigestible markers. Certain of these, in addition to being essentially indigestible by mammals (Bell, 1963; Garner *et al.*, 1960; Hamilton, 1947), become tightly bound to plant material (Morgan, 1959) and, therefore, might be expected to flow through the gastrointestinal tract in close association with indigestible feed residues. Such an associated flow would be desirable in reducing variation in fecal marker concentration attributable to differential flow of feed residue and marker from the reticulorumen (Corbett *et al.*, 1959).

Adsorptive effects by rare earth cations occur at concentrations approximating, or less than, the molar solubility of the corresponding hydroxides (in the order of 10^{-6} to 10^{-7} *M*) and at "radiocolloidal behavior" concentrations (below 10^{-6} to 10^{-7} and in the order of 10^{-11}*M*) (Kyker, 1961; Schweitzer and Jackson, 1952). These concentrations are below the detection range of the usual methods of chemical analysis and, hence, radioisotopes of the elements have usually been employed in studying such behavior. However, problems of disposal of large quantities of fecal waste are associated with the oral radioisotope administration to large animals. These problems of waste disposal may be circumvented by radioactivation analysis of those rare earth element(s) of sufficiently high nuclear cross section to provide the required analytical sensitivity in the feces of animals fed the nonradioactive isotope of the element. Dysprosium was selected on this basis as a representative of the rare earth elements. Its determination and some of its properties as an indigestible marker are the subject of this report.

MATERIALS AND METHODS

The required amount of dysprosium oxide (99.9% pure, Johnson, Mathey, and Co., Ltd., 73/83 Hatton Garden, London EC1) was dissolved by heating in 20 ml. of concentrated hydrochloric acid and diluted with water to 1 liter. Solutions made up for analytical purposes contained 0.3 or 2.0 μg. of dysprosium per ml. and were stored at a pH of approximately 1.5. Solutions for admixing with forage contained 1.362 grams of dysprosium per liter in which the pH was adjusted with solid calcium hydroxide to 3.5. The latter solution was sprayed over 227 kg. of ground hay as it was being mixed in a vertical auger mixer.

Three mature wethers were fed twice daily (6:30 A.M. and 5:30 P.M.) an amount of the dysprosium-containing forage, which represented approximately 90% of a previously established *ad libitum* level of consumption. There were no feed refusals during the 25-day preliminary period and the 8-day fecal collection period. The animals were accustomed to the collection stalls and the experimental routine prior to the actual collection period. Feces were collected after the a.m. feeding, weighed, and dried at 75° C. in a forced draft oven. One animal (No. 3) began scouring on the fifth collection day and further collections from it were discontinued.

Subsequent to the regular fecal collection period, fecal samples were collected at several intermediate time intervals during a 24-hour period to provide data on diurnal variations in dysprosium excretion.

Radioactivation analysis for dysprosium was conducted using an automated instrumental system developed by the Activation Analysis Research Laboratory, Texas Engineering Experiment Station, Texas A&M University, described briefly by Wainerdi (1963) and in greater detail by Fite *et al.* (1961). This system regulated and recorded times associated with the automatic, successive pneumatic transfer of samples from storage to the irradiation field, to the detector, and, after the preset counting time, to storage. Integrated into this system was a 256 channel pulse height analyzer, a 5.08 × 5.08 cm. well-type sodium iodide scintillation detector, and live and real time units. Output data to a tape punch and tape-to-card converter consisted of sample identification, irradiation time, pneumatic transit time from irradiation field to detector, live and real count time, and counts in each channel. This system was located adjacent to a swimming pool–type reactor which served as the source of thermal neutrons at a flux of approximately 10^{11} neutrons per cm.-second. Typical operating conditions were: irradiation time, 1 minute; irradiation-to-detector transit time, 0.3 minute; and live count time, 100 seconds.

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These operating conditions were selected to minimize the reactor and integrated system time required per sample. The amount of radioactive dysprosium produced by 1 minute of irradiation was slightly less than one half of that possible, since an irradiation time equal to the half life of the produced radioisotope (1.3 minutes) would result in 0.5 of the saturation level. Thus increasing irradiation time beyond 2 to 3 minutes would not greatly increase the method's sensitivity but would greatly reduce the number of samples which could be processed.

A measure of the 0.108 m.e.v. peak area (Figure 1) was obtained by the method of Covell (1955) involving three channels (0.003 m.e.v. per channel) on either side of, and the photopeak channel. A dysprosium standard, similarly prepared and packaged and interspersed among unknown forage and fecal samples, was similarly irradiated and measured. The mass of the unknown was calculated using data obtained from the standard by the following formula:

$$M_{(u)} = M_{(s)} \cdot \frac{1 - e^{-\lambda T_{i(s)}}}{1 - e^{-\lambda T_{i(u)}}} \cdot \frac{A_{0(u)}}{A_{0(s)}} \cdot \frac{\Phi_{(s)}}{\Phi_{(u)}} \qquad (1)$$

where: M = mass in unknown (u) and standard (s); λ = decay constant for dysprosium (9.24×10^{-3}); T_i = irradiation time for unknown (u) and standard (s); A_0 = activity at end of irradiation; and Φ = neutron flux at irradiation site.

Variations between samples in transit time (T_w) from irradiation field to detector and in the live count time (T_{live}) to real time (T_{real}) ratio were particularly critical for the 1.3-minute half-life radioisotope, necessitating correction of induced radioactivity to a standard time (A_0) for each individual sample and standard. The observed count (C_{obsd}) was corrected to activity at the end of irradiation (A_0) by the following formula:

$$A_0 = \frac{C_{\text{obsd}}}{T_{\text{live}}} \cdot e^{\lambda}\left\{T_w + \frac{1}{\lambda} \cdot \ln\left[\frac{2}{1 + e^{-\lambda}(R_{\text{real}})}\right]\right\} \qquad (2)$$

Initial efforts were directed to irradiating unprocessed rumen contents or fecal dry matter. However, the smallest quantities of these materials which could be accurately measured or weighed produced excessive radioactivity for purposes of analysis by analytical gamma spectrometry. The resultant live count time to real time ratio was so low (less than 60%) that excessive decay occurred during a live time lapse of 100 seconds. This was not the case with the dysprosium-containing forage and may indicate that much induced radioactivity in rumen contents and feces was from sodium present in higher concentrations in the latter materials. This problem was circumvented by wet ashing 20 grams of rumen contents, 0.5 gram of feces, or 1 gram of forage with nitric-perchloric acid. The nitric-perchloric digests were diluted to 100 ml., mixed, and the insoluble ash allowed to settle. One-milliliter aliquots of the diluted digests were placed in snap-cap polyethylene vials for irradiation. Duplicate determinations were run for approximately one half of the samples, and single samples of the remainder were analyzed. Similar vials containing 0.3 μg. of dysprosium in 1 ml. of nitric-perchloric acid were run

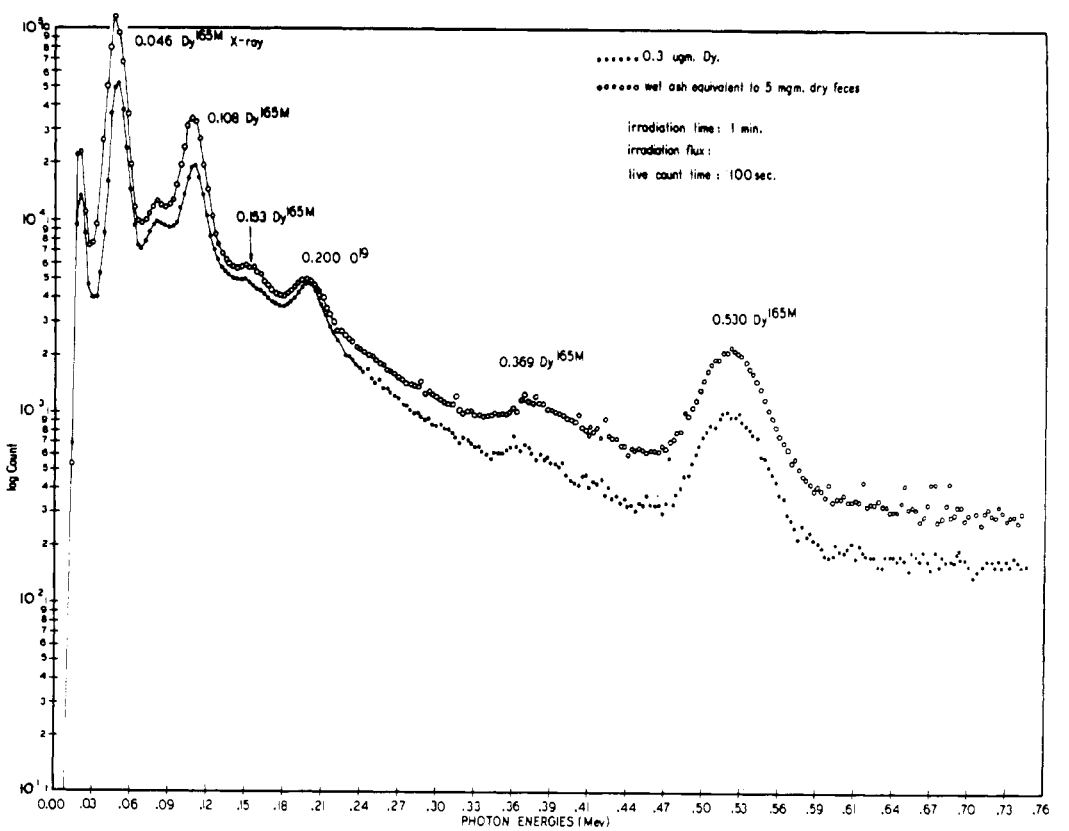

Figure 1. Gamma emission spectra of irradiated dysprosium standard (●—●) and the acid digest equivalent to 5 mg. of feces from sheep fed dysprosium (○—○)

with each 10 samples. Internal standards were similarly analyzed and were composed of 0.9 ml. of an unknown digest and 0.1 ml. of a nitric-perchloric acid solution containing 0.2 μg. of dysprosium.

The gamma emission spectra for a dysprosium standard and an aliquot of the nitric-perchloric acid digest of a fecal sample are reproduced in Figure 1.

An in vitro experiment was conducted to determine the distribution of dysprosium incubated with rumen contents, various fractions of rumen contents, or an electrolyte solution similar in composition to rumen fluid. The following were placed in 50-ml. centrifuge tubes: 10 grams of whole rumen contents, 10 ml. of rumen fluid obtained by centrifuging an aliquot of rumen digesta at 1800 × G for 10 minutes, or 11,000 × G for 15 minutes, and 10 ml. of "artificial saliva" (McDougall, 1948). Dysprosium (424 μg. in 0.5 ml.) was added to each tube and all tubes were incubated at 39° C. for 30 minutes. The tubes were then centrifuged at 11,000 × G for 15 minutes and the supernatant and residue separated by decantation. Each fraction was analyzed for dysprosium as described above.

RESULTS AND DISCUSSION

Measures of the analytical reproducibility of the method are given in Table I. Eight standards, interspersed among 95 samples and determined during three different series of analyses showed a coefficient of variation of 0.6% in the counts produced at the end of 1 minute's irradiation. Since this magnitude of variation could easily be accounted for by volumetric errors in dispensing the standard, variations in neutron flux from the reactor at the irradiation site (Φ) appeared negligible and were considered constant in all mass calculations (Equation 1).

The method employed here appeared to have an error of approximately 3%. Variations between duplicate aliquots of the digest of a single sample were large, approximately five times that observed for the standards. The reason(s) for this relatively large variation was not readily apparent. One possibility might be errors in sampling a nonhomogeneous digest, since insoluble materials were allowed to settle, and the digest was not remixed before sampling. However, the digest was sufficiently acid and the dysprosium concentration sufficiently low to avoid hydrolysis and the formation of insoluble hydroxides (Kyker, 1961).

The recovery of dysprosium when added to sample digests is summarized in Table II. The recoveries were, within the analytical errors involved, essentially 100%.

The quantity of dysprosium ingested was, within the analytical errors involved, quantitatively recovered in the feces (Table III). This is in agreement with reports that other elements of the rare earth group are essentially unabsorbed from the mammalian gastrointestinal tract. Quantitative fecal recovery has been reported for yttrium (Hamilton, 1947; Marcus and Lengemann, 1962), promethium (Hamilton, 1947), and cerium and praseodymium (Bell, 1963; DuBois, 1956; Garner *et al.*, 1960). Probably the most sensitive measurement was that by Bell (1963), who administered a large oral dose of radioactivity (20 mc.) as ^{144}Ce–^{144}Pr and could detect only 0.03% of the dose in extravisceral tissues and urine. Absorption of less than 0.05% was typical for yttrium and promethium (Hamilton, 1947). That lanthanum is not absorbed is also inferred from its toxicity when administered intraperitoneally and lack of toxicity when administered via oral routes (Cochran *et al.*, 1950).

Table I. Reproducibility of the Radioactivation Analysis Method Employed

Item	Relative Net Counts in Dy. Standard,[a] C.P.S.	Duplicate Samples of Forages and Feces, μg./Gram
Number of determinations	8	28[b]
Mean	906	120.5
Standard deviation	5.46	3.5
Coefficient of variation	0.6%	2.9%

[a] Corrected to c.p.s. at the end of irradiation, A_0.
[b] Number of samples for which duplicate aliquots of the digest were analyzed.

Table II. Recovery of Dysprosium When Added to the Nitric-Perchloric Acid Digest of Forage and Feces

Digest	Dysprosium, μg./Ml. Initially determined	Added	Total determined	Recovery μg.	Recovery %
Forage	0.282	0.200	0.489	0.207	103.5
Feces	0.580	0.200	0.785	0.205	102.5
Feces	0.562	0.200	0.757	0.195	97.5
Mean					101.2

Table III. Fecal Recovery of Ingested Dysprosium

Sheep No.	Collection Period, Days	Period Dy. Intake P.p.m.	Period Dy. Intake Mg.	Period Dy. Excreted P.p.m.	Period Dy. Excreted Mg.	Recovery, %
1	8	36.2	188.02	120.50	183.40	97.5
2	8	36.2	188.02	120.80	193.28	102.8
3	4	36.2	88.87	88.07	88.07	99.1
Mean						99.8

These results and a consideration of the similarity in their chemical properties (Kyker, 1961) suggest the rare earth elements as a group to be indigestible and permit considerable freedom in selecting the element or isotope most suitable for a particular application or analytical capability. Dysprosium was selected for the present investigation because of its applicability (high nuclear cross section for thermal neutrons, 2700 barns, and short half life which permitted rapid buildup of the radioisotope) to the high speed, automated instrumental radioactivation analytical facilities available. However, owing to its short half life, dysprosium might pose problems for a less integrated, automated facility. Alternatively, europium may be more desirable than dysprosium where less automated facilities are available. Europium has a high nuclear cross section (2500 barns for ^{151}Eu) and reacts with thermal neutrons to produce high energy gamma emitting radioisotopes (^{152}Eu and ^{154}Eu) having relatively long half lives (5.3 years).

Other than dysprosium and europium, none of the other rare earth elements appears well suited for instrumental radioactivation analysis (owing to the lack of gamma-emitting radioisotopes) at the levels employed here (owing to low nuclear cross section).

Variations in the concentration of dysprosium in representative samples of forage and the resultant feces are summarized in Table IV. Variations in the dysprosium content of the forage were represented by a coefficient of variation of 3.4%. This was only slightly larger than the coefficient of variation associated with the determination of dysprosium (2.9%, Table I), and suggested that most of this variation could be attributed to total analytical error of the method, and that dysprosium was very uniformly distributed on the forage. In general, daily variation in fecal concentration of dysprosium appeared to be approximately twice that of the variation in its concentration in the forage intake (Table IV). Within-day variation in fecal concentration of dysprosium (Table III) was of a similar magnitude as between-day variation for one sheep (No. 2) and similar to its variation in forage for the other sheep (No. 1).

Table IV. Variation in Daily Concentration of Dysprosium in Forage and Feces

	μg./Gram of Dry Matter			
		Feces from Sheep		
Date	Forage	1	2	3
6-21	36.2	119.9	117.9	110.6
6-22	36.1	108.5	125.2	108.9
6-23	37.2	140.0	124.6	97.9
6-24	34.2	140.6	122.3	116.6
6-25	34.8	133.2	103.4	
6-26		125.5	118.8	
6-27		125.7	115.4	
Mean	35.7	127.6	118.2	108.5
Standard deviation	1.2	11.4	8.0	7.8
Coefficient of variation	3.4	8.9	6.7	7.2
6-27, 6:30 A.M.		127.9	101.3	
6-27, 8:30 A.M.		126.9	123.0	
6-27, 12:30 P.M.		126.4	113.1	
6-27, 5:30 P.M.		127.7	118.6	
6-27, 9:00 P.M.		130.1	130.2	
6-28, 6:30 A.M.		117.6	125.5	
Mean		126.1	118.6	
Standard deviation		4.3	10.4	
Coefficient of variation		3.4	8.7	

Collectively, these data indicate variations in concentrations of dysprosium within and between daily samples of feces of between two and three times the analytical error involved in its determination. This order of fecal marker variation is lower than that generally reported for water-insoluble markers such as chromic oxide when admixed with the diet of sheep (Elam *et al.*, 1962; Kameoka *et al.*, 1956). A major source of such variation in fecal chromic oxide excretion has been attributed to a dissociated flow from the rumen by feed residues and marker (Corbett *et al.*, 1959).

Morgan (1959) reported that ^{144}Ce, derived from simulated fall-out, was tenaciously adsorbed onto forages and not subsequently removed by repeated rain washing. Such an adsorptive effect might be expected to occur in the rumen, and could contribute to reducing any dissociated flow by marker and forage residues out of the rumen, and thereby minimize variations in resultant fecal marker concentration. However, such adsorptive effects from solutions are thought to be related to the colloidal or radiocolloidal behavior of rare earth cations usually cited to occur at concentrations of approximately 10^{-7} to $10^{-11}M$, respectively (Kyker, 1961). The concentration of dysprosium in rumen contents was calculated to be in the order of $10^{-5}M$ in the present in vivo experiments and was thus in greater concentration than usually associated with adsorptive properties. However, a number of factors in addition to, and interactive with, concentration have been demonstrated to influence the degree with which other rare earth cations are adsorbed. Although no specific data could be found for dysprosium, it can be presumed that the following would also apply to dysprosium.

Schweitzer and Scott (1955) presented data indicating that the amount of yttrium adsorbed from solutions ranging from $10^{-4}M$ to tracer (10^{-10} to $10^{-11}M$) increased exponentially with increasing pH and adsorbent (Norit A carbon) concentrations. The maximum adsorbent concentration studied was 100 mg. per 5 ml. (2%), which resulted in an adsorption of over 90% of the yttrium from a $10^{-4}M$ solution at pH 6. Rumen contents are of a similar pH, contain from 10 to 15% of dry matter and, if other factors are not significantly involved, a similar degree of adsorption by dysprosium at $10^{-5}M$ might have been maintained in the rumen. However, this suggestion was not directly supported by the results obtained from the in vitro experiment (Table V).

Dysprosium was completely deposited as sediment from the artificial saliva media at a concentration of $2.6 \times 10^{-4}M$. Since the artificial saliva media contained no precipitable adsorbent, sedimentation presumably occurred by hydrolysis to the highly insoluble dysprosium hydroxide. Had complete adsorption occurred in the 11,000 × G rumen digesta centrifugate media, which would contain adsorbent, significant amounts of dysprosium should not have been precipitated by a repeated force of 11,000 × G after incubation with dysprosium. The high proportion of

Table V. Distribution[a] of Dysprosium[b] after Incubation with Whole Rumen Contents, Fractions of Rumen Contents, or Artificial Saliva

	Incubation Media			
		Whole Digesta Centrifugate		
Item	Whole digesta	1800 × G	11,000 × G	Artificial saliva
Media pH	5.8	6.3	6.5	6.4
Centrifugate	0.4	1.0	1.5	Nil
Sediment[c]	99.6	99.0	98.5	100%
Total Dy. recovered, %	98.0	90.3	98.1	92.7

[a] Distribution within a fraction as a per cent of the total determined in the two fractions. [b] Dysprosium concentration of $2.6 \times 10^{-4} M$. [c] Sediment obtained by 11,000 × G for 15 minutes.

dysprosium sedimentation from the 11,000 × G centrifugate and the similar proportion precipitated from the digesta in the 1800 × G fraction media suggest that, if adsorption of dysprosium onto forage residues occurred in the in vitro ruminal environment, it must have occurred at concentration considerably below $10^{-4} M$.

Previous mention has been made of the binding onto ryegrass by cerium from simulated fall-out (Morgan, 1959). The rapidity and tenacity of the binding in the experiments of Morgan was indicated by the high recovery of "fall-out" cerium on the forage, in contrast to soil, and its adherence to the forage during subsequent rains. This might suggest that forage-bound rare earth cations would be unavailable to undergo reactions available to the rare earth chloride in the in vitro ruminal environment. This dissimilarity in experimental conditions between the in vitro and in vivo experiments reported here limits the conclusions which can be drawn from the in vitro experiment concerning the in vivo experiments.

A further possibility, not tested here, is that if dysprosium hydroxide was formed in vivo, as it appeared to be in the artificial saliva media, it might have remained in colloidal dispersion or as protected colloids (Kyker, 1961) around the forage particles, which previously adsorbed the dysprosium ion. Such colloids may have been sufficiently dense to have been precipitated by the forces employed in the in vitro experiment but remain in intimate association with the forage residues in vivo.

The results of these experiments do not provide direct evidence supporting or refuting the hypothesized adsorption of dysprosium onto the forage fed or the resultant residues during their transit in the gastrointestinal tract. They do confirm the value of dysprosium as an indigestible marker and demonstrate the applicability of instrumental radioactivation analysis as a means for its rapid determination in fecal material. The low within- and between-day variation in fecal dysprosium concentration suggests potential advantages for rare earth elements over other presently accepted indigestible markers. Direct evidence to support this has been obtained in this laboratory (Huston and Ellis, 1965). Other applications might include the use of dysprosium (or other rare earths) as an external marker for range or pasture vegetation to qualitatively and/or quantitatively measure consumption. Different rare earths could be applied to different forages, which would bind them tenaciously (Morgan, 1959), and from radioactivation analysis of the different forages and the resultant feces, their relative and absolute consumption by grazing animals could be calculated.

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