fraction, appears to be normally only approximately 30-40% saturated. In Vit. B_{12} deficiency the unsaturated binding capacity is normal but total binding capacity is diminished. 2. It is postulated that the slower than normal disappearance of Co^{57}-B_{12} from the plasma of B_{12} deficient individuals is caused by diminished binding capacity in the tissues.

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Effects of Hydrobiotites upon Strontium-89 and Cesium-137 Retention By Ruminant Animals.* (28909)

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The literature is well documented with reports concerned with the movement and physiological behavior of radio-strontium and cesium(1-4), and extensive data are available on various dietary and therapeutic measures for limiting absorption and deposition in monogastric animals(5-7). However, there is little information on complexing agents suitable for use with ruminant animals. In that more than half of our added body burden of radioactivity originates from milk and milk products, and another one-fourth is derived from meat and animal products(8-9), there is a potential need for a practical procedure for safeguarding this portion of our food-chain against chronic ingestion of low levels of radioactive materials. Several reports(3,10-12) have indicated various degrees of success with commercial vermiculite for influencing excretion routes, and for decreasing skeletal retention of Sr-89 and of Cs-137. Initial studies in this laboratory(13) have revealed interesting possibilities for special processed vermiculite ores for selective sorption of these cations in the rumen for subsequent excretion, unabsorbed.

This study was initiated, therefore, to investigate the binding qualities for Sr-89 and Cs-137 of a refined vermiculite, verxite, a nearly pure exfoliated hydrobiotite, *in vivo* and *in vitro*, in an effort to prevent gastrointestinal absorption of these cations in the ruminant animal.

Methods and materials. The *in vitro* studies consisted of incubating tracer levels of Sr-89

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or Cs-137 with various concentrations of treated vermiculite ores under controlled conditions, *e.g.*, time, temperature and pH, and calculating the percent of radioactivity retained by the ore. One to 5 μc of radioactivity were added to 25 ml solution in 50 ml Erlenmeyer flasks, and weighed quantities of the ore were then mixed into the solution. Following incubation, with shaking, for various periods of time at room temperature and at 37°C, samples were centrifuged and 3-4 ml aliquots taken for radioactivity measurements, and for calculation of percent sorption by the ores.

The *in vivo* investigations consisted of 7-10 day blood-balance studies with yearling wether lambs maintained on adequate rations of cotton seed hulls, soybean oil-meal and ground corn, plus 3 or 5% of the treated ore, 2 weeks before oral or intravenous administration of a single tracer level of Sr-89 or Cs-137. Calcium level approximated 1% of the ration with a calcium to phosphorus ratio of 1.8. Before dosing with radioactivity, animals were placed in individual metabolism units, equipped for quantitative separate collection of urine and feces(14). The radioactivity in the periodic blood and ashed excreta samples was measured by conventional procedures using a sodium iodide crystal attached to a scaler unit, and the percent dose calculated, using standards prepared from the solutions adminsitered. It should be pointed out that the difficulty of quantitative eluding the radioactivity from the excreted hydrobiotite necessitated taking several small fecal samples at each collection period and ashing separately for analysis, rather than using conventional aliquots of large samples.

Results. In vitro. Preliminary studies showed that when 3 μc of Sr-89 were incubated with shaking in a 50 ml flask containing 25 ml of distilled water or 0.1 N NaCl, NaOH, KOH or HCl for 3 hours, there was no special advantage in adding more than 2-3% treated (phosphated) verxite. Sorption of Sr-89 was decreased, however, by NaCl and by NaOH, indicating sodium ion inhibition (15), and sorption was significantly reduced by the low pH of the acid solutions.

TABLE I. Strontium-89 Removed from Solution by Native and Treated Vermiculite Ores.*

Treatment	Native ore	Activated ore	Expanded ore
Distilled H_2O	70 ± 3†	88 ± 4	84 ± 4
.1 N NaCl	54 ± 2	58 ± 2	61 ± 3
.1 N NaOH	52 ± 2	61 ± 3	64 ± 2
.1 N KOH	60 ± 3	80 ± 4	80 ± 5
.1 N HCl	8 ± 1	10 ± 1	12 ± 1

* Solutions incubated 3 hr, 37.5°C with 3% ore.
† Calculated as mean % retained by added ore, ± standard deviation.

Data in Table I summarize an average of 30 samples per treatment per ore and show the effects of ore treatment upon sorption of Sr-89 from these various solutions containing 3% of the verxite by weight. Native ore was the least effective, while the activated (phosphated) and the expanded or exfoliated ores showed the highest retention properties. It is again evident that sorption was enhanced by potassium ions(16), and reduced by sodium, and by the lower pH solutions.

The relative effects of various treatments upon Sr-89 and Cs-137 sorption by the activated ore added at the 3% level to 325 samples are shown in Table II. After 3 hours' incubation, significantly more Cs-137 than Sr-89 was removed from all solutions, and Cs-137 appeared to be less dependent upon solution pH. However, sorption of both cations was increased in the neutral and alkaline solutions, and potassium ions appeared to enhance the quantity removed from solution (17). When incubated with juices from the gastrointestinal tract of ruminants—rumen, stomach and small intestines—significantly

TABLE II. Strontium-89 and Cesium-137 Removed from Solution by Verxite (Activated).*

Treatment	% cation removed† Strontium-89	Cesium-137
Distilled water	84 ± 5	95 ± 5
.1 N NaCl	58 ± 3	77 ± 4
.1 N NaOH	65 ± 4	82 ± 6
.1 N KOH	80 ± 4	90 ± 5
.1 N HCl	10 ± 1	75 ± 3
Rumen juice	35 ± 4	85 ± 7
Stomach cts	10 ± 1	55 ± 3
Small intestine cts	25 ± 1	58 ± 5

* Solutions incubated with shaking, 3 hr at room temperature with 3% Verxite (activated).
† Calculated as mean % retained by ore ± standard deviation.

TABLE III. Effects of Dietary Verxite (Activated) upon Cesium-137 and Strontium-89 Excretion by Yearling Wethers.

Treatment	No. trials	Dose* MOA	% dose excretion† Feces	Urine	Total
Cesium-137					
Controls	8	OS	32	35	67 ± 8
	4	IV	27	36	63 ± 6
Verxite, 5%	8	OS	53	41	94 ±12
	4	IV	42	45	87 ±10
Strontium-89					
Controls	3	OS	72	10	83 ±10
	3	IV	44	29	73 ± 9
Verxite, 5%	3	OS	84	12	96 ±10
	3	IV	47	40	87 ± 7

* Animals administered single tracer dose Cs-137 or Sr-89, intravenously (IV) or orally (OS) by stomach tube.

† Mean % administered dose excreted in feces, urine, and total (7-day balance) excreted ± standard deviation.

more of the Cs-137 was removed by the ore, and again pH appeared to have a greater effect upon Sr-89 sorption. Physically and chemically then, the high pH of the rumen would simulate conditions highly favorable for maximum sorption of these cations *in vivo*.

Results from feeding treated ores to ruminants. Early investigation with growing lambs indicated that when verxites were incorporated into a cottonseed hull, soybean oil meal and corn ration at either 3 or 5% levels, there were no apparent adverse effects upon feed intake or growth during 8-10 weeks feeding trials. When incorporated as 10% total ration of milk cows for 8 weeks by Wisconsin workers no reduction in milk flow or other unfavorable changes in animal status were observed. The results of feeding 0 and 5% verxite upon Sr-89 and Cs-137 excretion in 36, seven-day balance studies with 24 yearling wethers are shown in Table III. Animals maintained on these rations for 2-4 weeks were placed in individual metabolism units and dosed either orally or intravenously with a single tracer level of 150 mcC Cs-137 or 200 mcC Sr-89 and blood-balance studies initiated.

Calculated excretion data indicate a marked decrease in retention of orally administered Cs-137 ($p<0.1$), and an increase in fecal excretion of the intravenously administered dose. Effects upon urinary excretion were not significant, but treatment appeared to increase endogenous loss of radiocesium from both the oral and intravenous doses. Total excretion, therefore, was significantly increased when 5% verxite was incorporated into the adequate rations of lambs.

When Sr-89 was administered orally to wether lambs the greater excretion occurred between 36 and 96 hours after dosing, and ultimately 13% more of the radio-strontium administered was excreted in the feces of the treated animals. Urinary excretion by orally dosed lambs was not significantly affected by verxite feeding; however, following intravenous administration, urinary Sr-89 was markedly increased. Urinary volume was not augmented by the treatment. Reasons for this effect, other than the observed increased endogenous losses, (Fig. 1), upon urinary Sr-89 excretion require further study. It is evident, however, that 84-106% of the ingested nuclide was recovered in the feces and urine of those lambs maintained on rations containing 5% dietary verxite. Results of 12 balance studies with lambs on similar rations plus 10% added verxite were not significantly different from these values. Data therefore were not separately presented.

Average Sr-89 values for the total blood of yearling wethers, as a function of time in hours after dose administration, are shown in Fig. 1. Total blood volume was calcu-

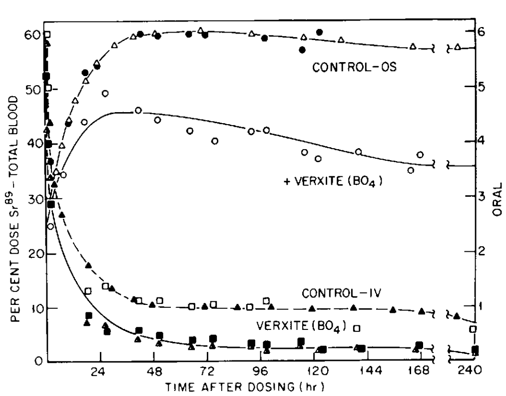

FIG. 1. Effects of dietary hydrobiotites (Verxite) upon orally (top) and intravenously (IV) administered Sr-89 in total blood of wethers as a function of time after dosing.

lated as 6.2% of the total body weight(18), and blood concentration values were employed to calculate total circulating Sr-89. The curves from those animals dosed intravenously indicated that the feeding of hydrobiotites increased the rate of Sr-89 loss from the total blood, and ultimately less was retained.

Following oral administration, the blood curves for the control lambs reflected a more rapid increase in absorption, reaching a peak of 6% after 36 hours, and maintaining this relative level to 240 hours, whereas, those animals receiving rations containing the verxite absorbed less (4.5%), and demonstrated a pronounced drop in total circurating radiostrontium after 47 to 72 hours. These values reflected not only decreased absorption, but indicated a marked increase in endogenous Sr-89 loss as previously observed (Table III). Blood curves following oral and intravenous dosing with radiocesium were quite similar to those for Sr-89 and, therefore, are not included here.

Discussion. Man's exposure to worldwide "fallout" is mainly through radioactive contamination of food and water, the radioactivity then being consumed and incorporated into the body where it serves as a source of internal radiation(2,12). Radio-strontium and cesium are the cations of most concern, because of their long half-lives they are cumulative toxins. Strontium is a "bone seeker" and a dangerous contaminant of soils, plants and animal products. Cesium is of somewhat less concern only because it is more rapidly bound in soil, and turnover rate of deposited cesium is more rapid(2).

Various approaches have been made to possible reduction or prevention of body contamination from the food supply(2,10,12,16, 17) by these cations. Chelates, binding agents and stable isotopes have been employed with little success in man or monogastric animals. *In vitro* data reported here indicate that the high acidity of the stomach is a contributing factor to these results. The rumen of the cow and the sheep, however, simulates more favorable conditions for binding of these cations for subsequent excretion, unabsorbed, although some radioactivity is either not bound or is eluded as it passes through the stomach itself (Fig. 3), as indicated by observed levels in the circulating blood. These points are being investigated.

Vermiculite ore was used in this study because of its inert chemical nature, practical economy and capacity for physical change by treatment for increased cation sorption. It is highly porous, having a low density and a cation exchange capacity of 20 to 30 milliequivalents per 100 g(19). Through pretreatment with heat, (expanded or exfoliated), acids (phosphated or activated) or by grinding, capacity may be altered significantly. Exfoliation of these silica-tetrahedral sheets results in larger pores being formed between the groups of platelets, increasing void volume without reduction in surface area, thus increasing capacity for cation capture(19). This ion exchange capacity and crystal structure allow these substances a strategic position, since even the cation exchange sites on the internal surfaces may be employed for binding(15). The fact that these ores are nontoxic and appear effective at low concentration and dietary levels makes them of practical value for use with ruminant animals for binding and thus reducing absorption of these hazardous cations from "fallout"(13). It is evident (Table III) that both exogenous and endogenous cations are affected by these dietary verxites, indicating a possible therapeutic value in that portion of the gastrointestinal tract where pH is favorable. These data further point out the fact that binding may occur throughout the tract, except for the stomach, and provide evidence that "fallout" levels of these radioactive contaminants could be effectively bound and subsequently excreted. More data are required concerning factors affecting binding, interrelationships and various other ration effects upon retention of these and related cations by the verxites. Furthermore data on effects of extended feeding periods upon the nutrition of growing and lactating animals, with special reference to calcium and potassium behavior, are required before hydrobiotites can be generally practical as a potential safeguard in our food chain against chronic ingestion of low level radioactive contaminants. Studies underway with Ca-47 and K-42, however, should help clarify many of the essential ration adjustments required for long term periods.

Summary. Radiochemical studies with ruminant animals indicate that activated verxites, a nearly pure hydrobiotite, effectively bind Sr-89 and Cs-137 *in vitro* and *in vivo,* thus increasing excretion rate and quantity of these radio-nuclides injected or ingested at tracer levels.

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Experimental Hepatic Siderosis Following Portacaval Shunt.* (28910)

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Hepatic siderosis associated with human cirrhosis is well known(1,7,9). Recently attention has been called to the rapid accumulation of hepatic iron in a few cirrhotic patients following construction of a portacaval shunt for relief of portal hypertension(4,11, 12). It has been claimed that cirrhosis is a necessary condition, and that hepatic siderosis does not follow creation of a portacaval shunt for extra-hepatic causes of portal hypertension, such as portal vein obstruction(11). To determine experimentally whether shunting of the portal flow is responsible for hepatic iron deposition, end-to-side and side-to-side portacaval shunts were constructed in rats utilizing microsurgical techniques. The animals were then fed normal and increased amounts of iron.

Materials and methods. The experimental group consisted of 20 male Sprague-Dawley rats, having an average initial weight of 400 g. They were divided into the following groups:

I: Six animals were subjected to end-to-side portacaval shunts and fed Rockland Farms complete rat diet (0.019% iron) plus 3% ferric ammonium citrate.

II: Four animals were subjected to end-to-side portacaval shunts and fed Rockland Farms complete rat diet.

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