Hexacyanoferrates and bentonite as binders of radiocaesium for reindeer.

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Abstract: The effects of varying doses of caesium binders (Bentonite and several forms of iron-hexacyanoferrates) on radiocaesium accumulation in red blood cells and on radiocaesium transfer to urine and faeces were studied in feeding experiments with reindeer calves. The caesium binders were added to a ration of lichen (containing 9.5 kBq of $^{134}Cs+^{137}Cs$ originating from the Chernobyl accident) and fed together with a pelleted reindeer feed (RF-71) for 42 days. A 50% reduction in red blood cell radiocaesium concentration was obtained with a daily dose of 1 mg/kg body weigth of ammoniumironhexacyanoferrate (AFCF) and with 500 mg/kg of bentonite. Three mg/kg of AFCF or 2 g/kg of bentonite reduced both urinary excretion and RBC concentrations with more than 80%. It is concluded that iron-hexacyanoferrates, as a result of their high caesium binding capacity, are particularly useful as caesium binders for free ranging ruminants like the reindeer.

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Keywords: radiocaesium, reindeer, caesium-binder, prussian blue, hexacyanoferrates, bentonite, environment, fallout

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Sammendrag: Effekten av bentonitt og ulike typer jernhexacyanoferrater (Berlinerblått) på akkumulering av radioaktivt cesium i røde blodlegemer og på utskilling av radioaktivt cesium i urin ble undersøkt i foringsforsøk med reinkalver. Cesiumbinderne ble gitt daglig sammen med lav som inneholdt 9.5 kBq $^{134}Cs+^{137}Cs$ fra Tsjernobyl ulykken, og 1 kg reinfor (RF-71) i en periode på 42 dager. En daglig dose på 1 mg/kg kroppsvekt av ammoniumjernhexacyanoferrat (AFCF) reduserte radiocesiuminnholdet i blodlegemer med 50%, mens en dose pa 500 mg/kg bentonitt var nødvendig for å oppnå samme effekt. Tre mg/kg AFCF eller 2 g/kg/bentonitt var nødvendig for å oppnå mer enn 80% reduksjon i radiocesium konsentrasjonen i blodlegemer og i radiocesium utskilling med urinen. På grunn av de små daglige mengder som kreves er jern-hexacyanoferratene spesielt velegnete som cesiumbindere for beitedyr.

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Introduction

After the Chernobyl nuclear accident radiocaesium content of meat from reindeer in parts of Scandinavia increased far above the intervention levels (6 kBq/kg in Norway). Decontamination of live animals was required before marketing of reindeer meat. Feeding schemes utilizing uncontaminated feeds were immediately implemented, and caesium binders of the clay mineral type (zeolites and bentomite) were tested experimentally and used in practical situations (Åhman 1988, Åhman *et al.*; 1990.;

Hove & Ekern, 1988). From 1989 ammonium-iron-hexacyanoferrate (AFCF) replaced bentonite as the caesium binder in reindeer feeds in Norway. The major benefit of AFCF was a very high efficiency and specificity in binding radiocaesium and a high chemical stability in the gastrointestinal tract of ruminants (Arnaud *et al.*., 1988). Our study was undertaken to establish the relationship between daily doses of the caesium binders and the accumulation of radiocaesium in animals fed lichen contaminated by fallout from the Chernobyl nuclear accident. An abstract of parts of the work was presented earlier (Hove *et al.*, 1990a).

Materials and methods

Animals and feeding

Male reindeer calves born in May 1986 were used for the experiment which lasted from November 1986 until April 1987. The animals were obtained from semidomestic herds in Southern Norway, and had been accustomed to handling during a period of feeding before the experiments started. During the experimental period calves were kept indoors (10°C) and alternated every two weeks between metabolism cages allowing collection of faeces and urine and tie stalls on the floor where collection of excreta was not possible. The calves weighed 45-55 kg at the start of the experiment, and most animals lost 1-3 kg during the study.

Lichen (mainly *Cladina alpestris* and *Cetraria spp.*) with a radiocaesium concentration of 30±4 kBq/kg dry matter (DM) of $^{134}Cs+^{137}Cs$ was obtained from a heavily contaminated area in Vågå, S. Norway. The lichen was stored frozen. Material for a feeding period of about two months was thawed when required, and contaminating soil and other non-lichen constituents were sorted out. The lichen was mixed manually, weighed into portions in plastic bags, and again stored frozen. The calves received 0.67 kg of lichen (0.32 kg DM) daily, giving a radiocaesium intake of 9.5 kBq/d. In addition 1 kg of the pelleted reindeer feed RF-71+4% sodium bicarbonate added as a buffer (Jacobsen & Skjenneberg, 1979) was given.

Samples

Collection of faeces and urine was done twice weekly during the two weeks when the calves were in metabolism cages. Urine was weighed and 5 ml used for radiometry. The whole collection of faeces was dried, ground, and a 3-5 g sample was used for radiometry. Blood samples were taken twice weekly from the jugular vein. Plasma was separated, and 5 ml of the red cellfraction (RBC) transferred to counting vials. The hematocrit of the red cell fraction was used to correct for plasma dilution when the radiocaesium concentration of RBC was calculated.

Experimental

Two series of experiments were carried out.

In *Series 1* the accumulation of radiocaesium with time was studied in 4 calves to gain knowledge on the length of the period required to reach steady state conditions in urine and RBC radiocaesium concentrations. Lichen was fed for 72 days. The results showed that more than 90% of plateau levels of urinary radiocaesium excretion and about 75% of plateau levels of RBC radiocaesium concentrations were reached after 42 days. This time period was therefore considered satisfactory for the testing of the caesium binders in series 2. Two of the calves were from day 42 given 300 mg/d of AFCF to assess the effect on the caesium binder in animals already loaded with radiocaesium.

In *Series 2* the effects of different doses of caesium binders on the accumulation of radiocesium were studied. Hexacyanoferrates included Ammonium-iron(III)-hexacyanoferrate(II) from Riedel de Häen, Seelze, Germany, an insoluble prussian blue preparation (iron(III)-iron(III)-hexacyanoferrate(II), FeFCF1) from British Drug Houses, UK, and a similar compound (FeFCF2) produced as a pigment by WEB Kali Chemie, Berlin, Germany. AFCF was given in daily doses of 50, 150, 300, 700 and 1500 mg/d. The other hexacyanoferrates were only tested in the 300 mg/d dose. Finely ground, feed grade Wyoming bentonite was given in daily doses of 25, 50 and 100 g.

The animals were fed contaminated lichen and the caesium binder for a period of 42 days. Two calves were used per dose. The caesium binders were sprinkled onto the lichens. The hexacyanoferrates and the 25 g dose of bentonite were readily eaten, but the higher doses of bentonite were consumed more reluctantly. Feeding of 100 g of bentonite resulted in clogging of bentonite to the hairs of the muzzle and face of the calves.

Radiometry

Samples of urine, faeces and red blood cells were counted for ^{134}Cs and ^{137}Cs in a LKB ultro-gamma counter in vials containing 5 or 10 ml of sample. The level of detection for radiocaesium in RBC and urine was between 20-50 Bq/L with the counting times used.

Calculations

The build up of radiocaesium concentrations in RBC, urine and faeces C(t) with time (t) could be described by the formula

$$C(t) = P(1-e^{\div kt}) \qquad [1]$$

where P is the estimated equilibrium concentration and k is the rate constant for the exponential term. The time required to reach 50% of plateau levels (halftime for the exponential term) was calculated as $t/2 = \ln(2)/k$.

Results

Series 1. Radiocesium excretion in faeces and urine reached 90-100% of their plateau levels within 6 weeks (Fig. 1). Estimated plateau levels for urinary excretion was 15% of the daily ingested dose of radiocaesium. Fitting equation [1] to the measurements of urinary radiocaesium concentrations resulted in a halftime of 11 d (Fig. 1). Radiocaesium excretion via faeces reached a plateau of approximately 75% of the daily ingested dose, and the build up of radiocaesium activity was described by equation 1 with a halftime of 6 d (Fig. 1). Loss of radiocaesium in faeces and urine accounted for about 90% of the daily ingested activity during the last weeks of the experiment, indicating that some accumulation of radiocaesium in muscle cells and other intracellular compartments were still taking place. RBC radiocaesium had attained about 80% of the equilibrium level at 6 weeks, and continued to increase for 2-4 more weeks (Fig. 1). Estimated plateau for RBC activity was 5.5% of the daily dose per liter of cells, and the half time for the exponential describing the build up was 21 days.

When AFCF (300 mg/d) was added to the diet, faecal excretion of radiocaesium increased immediately to 110% of the ingested dose, demonstrating that a reduction in body load took place at the same time as the absorption decreased. After 4 weeks of feeding 300 mg/d AFCF faecal excretion decreased, probably as a result of a depletion of the body load of radiocaesium. Urinary excretion of radiocaesium declined by 85-90% compared to the animals which not were fed AFCF, and RBC concentrations of radiocaesium were reduced by about 90% in 3-4 weeks (Fig. 1).

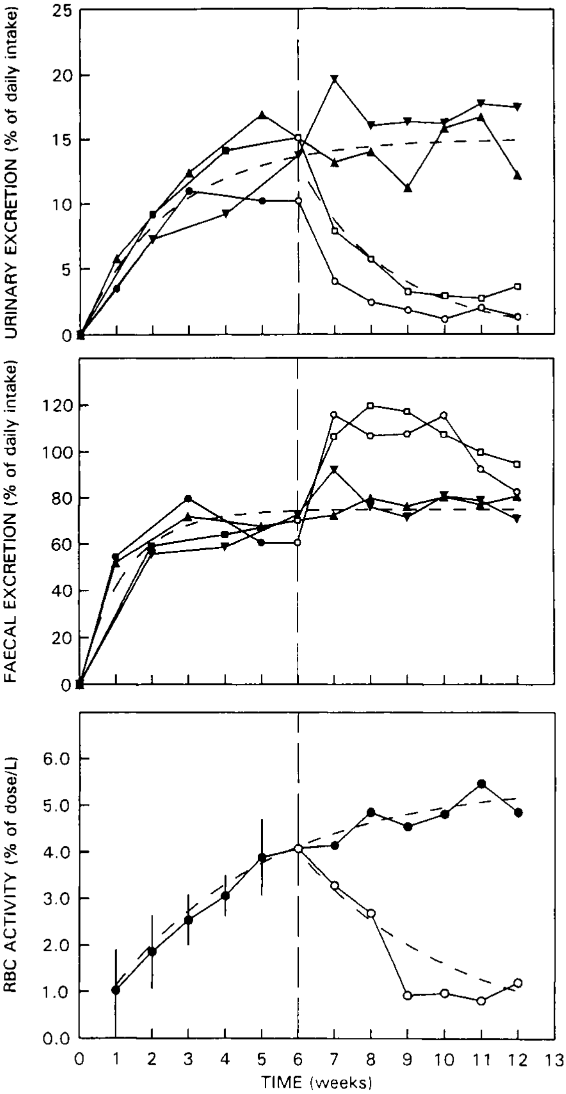

Fig 1. Change in urinary and faecal excretion of radiocaesium and in red blood cell (RBC) radiocaesium activity in reindeer fed 9.5 kBq/d of radiocaesium in contaminated lichen for 12 weeks (Series 1). After 6 weeks 2 animals were given 300 mg/d of ammonium-ironhexacyanoferrate (open symbols) while the two other received no caesium binder. RBC radiocaesium concentrations (bottom graph) are given as means and standard deviations during the period when the no caesium binder was given. Curves with broken lines represent the fitted exponential formulas for the build up of radiocaesium concentrations.

Series 2. A dose-dependant reduction both in urinary radiocaesium excretion (Fig. 2, Table 1) and in RBC concentrations (Table 1) was observed when bentonite was added to the diet in increasing amounts. Average reduction in urinary excretion ranged from 47% with the 25 g dose to 85% when 100 g bentonite was fed. RBC concentrations of radiocaesium during the last 3 weeks of the 6 week observation period were reduced with 35, 75 and 80% re-

Table 1. Radiocaesium excretion in urine of reindeer calves fed radiocaesium and given different daily doses of hexacyanoferrates or bentonite. The values given are averages of daily excretions of radiocaesium day 11-42 for urine and averages of values day 21-42 for red blood cells (RBC). The percent reduction was calculated relative to urinary radiocaesium excretion in the control animals.

		Urinary excretion		RBC	
Caesium binder	n	Bq/d	% reduction	Bq/L	% reduction
None (control)	4	1131±229	-	328±37	-
AFCF 50 mg	2	-	-	127	61
AFCF 150 mg	2	203	82	43	87
AFCF 300 mg	2	208	80	42	87
AFCF 700 mg	2	109	90	28	91
AFCF 1500 mg	2	122	89	22	93
FeFCFl 300 mg	2	198	83	58	83
FeFCF2 300 mg	2	246	78	59	83
Bentonite 25 g	2	602	47	210	36
Bentonite 50 g	2	381	64	81	75
Bentonite 100 g	2	168	85	75	78

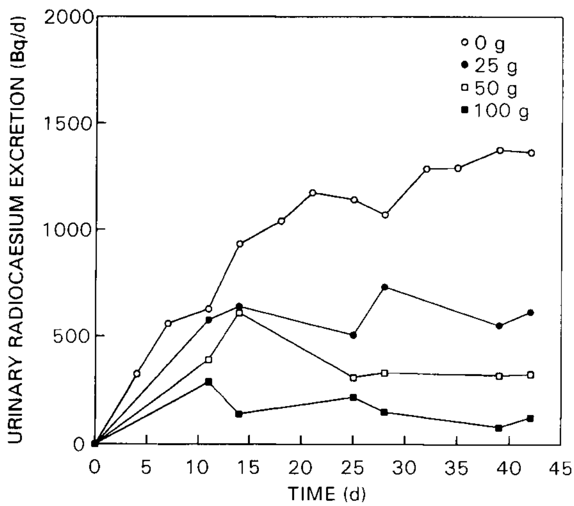

Fig. 2.Urinary radiocaesium excretion from animals fed 9.5 kBq/d of radiocaesium in contaminated lichen and different amounts of bentonite (Series 2, mean of two animals per dose level of bentonite). The curve for the control animals (0 g) was constructed from observations in series 1 of four animals sampled at different times. Each data point is the average of observations from two animals.

spectively after feeding 25, 50 and 100 g/d of bentonite (Table 1).

Mean RBC radiocaesium concentrations day 21-42 were reduced by 60% with 50 mg/d of AFCF increasing to 87% and to 93% with the doses of 150 to 1500 mg/d (Table 1). Radiocaesium concentrations in RBC were 59 and 57 Bq/L when FeFCF1 and FeFCF2 were fed. These values were very close to the individual averages of 40-50 Bq/L observed for the 150 and 300 mg doses of AFCF (Table 1). The same dose dependent reductions were found in urinary excretion of radiocaesium (Table 1). In urine the reduction was significantly greater for the two highest dose levels than for the 150 and 300 mg/d doses. No difference in the level of reduction was detected between the three different hexacyanoferrate preparations when tested at the 300 mg/d dose level.

Discussion

The experimental period for testing the efficiency of the caesium binders was 42 days. Analysis of the kinetics of radiocaesium excretion showed that an equilibrium in the excretion of radiocaesium in urine and faeces had probably been attained during the period from 21-42 d. Blood radiocaesium activity was however still increasing (Fig. 1). From other studies (Pedersen, Staaland & Hove, unpublished) it can be inferred that also the meat would accumulate radiocaesium after 42 days of feeding of contaminated lichen. In calculating the levels of reduction for the various treatments more precise estimates could probably have been reached by prolongation of the experimental periods, but considerably longer feeding periods would have been neccessary to obtain equilibrium in slow turnover pools like muscle cells.

Hexacyanoferrates have for long been recognized as effective caesium binders reducing radiocaesium accumulation in a wide variety of animals including rats (Nigrovic., 1963), pigs (Giese., 1970; Rudnicki, 1988) and several ruminant species like the goat (Havlicek *et al.*, 1967; Hove *et al.*, 1990b), cow (Arnaud *et al.*, 1988; Giese, 1988) and reindeer (Hove *et al.*, 1988; Staaland *et al.*, 1990). The early studies with prussian blue used the iron(III) and the potassium complexes of the hexacyanoferrates. Giese & Hantzsch (1968) and later Nielsen *et al.* (1987) reported a somewhat superior efficiency for the ammonium salt when compared to the alkali hexacyanoferrates in vitro. Only AFCF has been used in Norway after the Chernobyl accident.

The doses of AFCF used in animal experiments have recently been reviewed by Giese (1989). In dairy cows doses of 3 g/d reduced milk radiocaesium with 80-90% and meat radiocaesium with 75%. In small ruminants like sheep and goats 1-2 g/d (20-40 mg/kg) were recommended by the manufacturer. Our experiments showed a significant reduction in radiocaesium transfer with as little as 50 mg/d (1 mg/kg body weigth). A dose dependent reduction in urinary radiocaesium excretion was observed up to 700 mg/d, while the the RBC reduction was maximal already at the 150 mg (3 mg/kg) dose level (Table 1). In agreement with this Mathiesen *et al.* (1990) reported that 250 mg/d AFCF prevented accumulation of radiocaesium. This effect of AFCF in dose levels much lower than those recommended is clearly beneficial when the caesium binder is used for free-ranging animals where daily feeding of concentrates is impractical. The efficacy of the preparation in small doses has enabled the use of AFCF as a countermeasure for grazing animals in the form of salt licks impregnated with 2.5% AFCF and in sustained release boli (Hove *et al.*, 1990a,b).

No controlled comparison seem however to have been performed on the action of the different chemical forms of the hexacyanoferrate binders in ruminants. The special features of the ruminant forestomachs with a large liquid volume and a long retention time which allow good contact between radiocaesium and the binder may allow for differences in the relative effect of the binders compared to monogastric species. The amount of caesium recycling to the rumen with saliva is also very large (Staaland *et al.*, 1990). In our study no significant difference between the effect of the studied forms of the hexacyanoferrates could be found at the 300 mg/d dose level (Table 1). This dose level was however not the most sensitive, since the level of reduction of radiocaesium in urine was already 80-90%, and further studies should be carried out with lower daily doses of the various hexacyanoferrate forms to adress this question.

Bentonite has been used for reduction of the transfer of radiocaesium from feed to animal products in both reindeer and domestic ruminants after the Chernobyl accident. In reindeer Åhman *et al.* (1990) reported about 80% reduction in whole blood ^{137}Cs levels when feeding reindeer either 23 or 46 g bentonite per day during a 21 day feeding trial. Simultaneously the urinary excretion was reduced by almost 90%. Such levels of reduction was only obtained with the 100 g dose levels in the present study. An explanation to this may be that Åhman *et al.* (1990) used a concentrate feed with bentonite which may have prevented the clogging of the preparate which was observed when the bentonite was fed as a powder. Differences in the caesium binding capacity of the bentonite preparations could however not be precluded.

The dose dependent reduction observed in radiocaesium transfer in the present study agrees well with results in dairy cows. Hove & Ekern (1988) observed 33, 77 and 68 % reduction in the radiocaesium transfer to milk when 250, 500 or 750 g bentonite was fed with contaminated hay, and Mitchell *et al.* (1989) reported 37 and 67% reductions in radiocaesium transfer to milk on the two lowest dose levels. Results from comparable dose/response trials appear not to be available for sheep, but reductions of 80% or more after feeding daily doses of about 50g was reported both by Van den Hoek (1976) and Anderson (1989), and Daburon *et al.* (1991) reported 60 and 85% reduction when sheep were fed 30 or 60 g/d of vermiculite respectively.

Caesium binders are added to the feed which is given to reindeer, sheep and cows during the special feeding programs undertaken to reduce the radiocaesium content of the meat to below the intervention limit. This addition is chosen to assure a minimal absorption from soil or from lichen fed to maintain appetite and optimal rumen function. Judged from studies in other ruminant species increased rates of radiocaesium loss from the body would hardly be expected in contaminated animals fed uncontaminated feeds (Bailer, 1988; Hove & Ekern, 1988; Philippo *et al.*, 1988). The present study shows that hexacyanoferrates will reduce radiocaesium accumulation by about 50% even in doses as low as 1 mg/kg body weigth. This high efficiency makes the hexacyanoferrates particularily well suited for reducing radiocaesium levels in grazing reindeer and other ruminants.

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