Synthesis, effectiveness and metabolic fate in cows of the caesium complexing compound ammonium ferric hexacyanoferrate labelled with ^{14}C

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SUMMARY. Adding ammonium ferric hexacyanoferrate (AFCF) to cows' fodder produced after the Chernobyl nuclear accident prevented milk contamination by increasing the faecal elimination of ^{137}Cs . Synthesis of ammonium ferric hexa[^{14}C]-cyanoferrate (AF ^{14}CF) and its purification were performed for the study of the metabolic fate of this complex, and the evaluation of the possible release of cyanide. The stability of this colloidal product, tested by anaerobic incubation in rumen juice *in vitro*, showed no release of free cyanide from AF ^{14}CF , but hexacyanoferrate was identified in the rumen juice and 0.13 % of the added radioactivity was converted to labelled CO_2 . AF ^{14}CF administered *per os* to two cows showed a nearly quantitative excretion of radioactivity in faeces during the first 3 d (91–95 %). A very low but significant level of radioactivity appeared in plasma, blood cells, expired CO_2 and was detected in organs taken 9 d after administration. Total cumulative radioactivity in urine and milk amounted to 0.19–0.47 % and 0.068–0.071 % respectively for the two cows. Labelled hexacyanoferrate and thiocyanate were identified in the urine and also in faeces. In spite of this relative instability of AFCF in the rumen of cows, the poor absorption of AF ^{14}CF degradation products showed that AFCF constitutes an efficient and safe food additive to prevent the absorption of radioactive caesium from ruminant feed and its secretion in milk.

The Chernobyl nuclear accident led to widespread contamination with the three main radioisotopes: ^{131}I , ^{137}Cs and ^{134}Cs . Iodine disappeared rapidly from the environment and food chain because of its short half-life of 8.04 d. Caesium contamination, however, mainly because the half-life of ^{137}Cs is 30 years and that of ^{134}Cs is 2 years, will take several years to return to the level observed before the nuclear accident. Consequently, it is necessary to investigate methods to reduce caesium concentrations in food and particularly milk and milk products which are to be used by infants, the most susceptible group in the human population. In addition,

as no internationally accepted level of radioisotopes in food is as yet recognized, the substantial reduction of radioactive caesium (radiocaesium) should produce a level of contamination within the most restrictive limits.

It has been shown that several salts of hydroferrocyanic acid can bind to radiocaesium when simultaneously administered orally to the rat (Nigrović, 1963, 1965; Nigrović *et al.* 1966; Madshus *et al.* 1966; Richmond & Bunde, 1966; Brenot & Rinaldi, 1967; Havlíček *et al.* 1967; Havlíček, 1968). Giese & Hantzsch (1970) demonstrated that AFCF was the most efficient for increasing the excretion of ^{137}Cs when given orally to rats, compared with alkaline iron cyanide complexes such as Li-, Na-, K-, Cs- and Rb-salts. Previously $\text{KFe}^{3+}[\text{Fe}^{2+}(\text{CN})_6]$ had been used as an additive to caesium-contaminated milk fed to pigs which resulted in large amounts of the daily oral radiocaesium intake being excreted with the faeces (Giese *et al.* 1970). Following this work, Nezel (1970) investigated the effect of AFCF in hens, where the ^{137}Cs uptake from contaminated feed was almost completely inhibited, and Giese (1971) tested it in cows fed milk containing added ^{134}Cs . There has been a renewed interest in AFCF and similar complexes following the Chernobyl nuclear accident and they have been proposed as an additive to cow fodder collected in areas where caesium contamination was high.

The enhanced excretion of radiocaesium in faeces might be explained by the stability of AFCF in the rumen and in the gastrointestinal tract, the stability being underlined by the absence of toxic effects of these complexes. To be able to apply this product as an animal food additive, it was necessary to evaluate quantitatively its stability *in vivo* and particularly the possible release of cyanide, its absorption, metabolism and elimination in urine and milk. Such data have not been reported to date.

This paper describes the novel synthesis of ^{14}C -labelled AFCF (AF^{14}CF), its purification and its metabolic fate in the cow and also reports the efficiency of AFCF in preventing contamination of milk by complexing the radiocaesium present in cow fodder.

MATERIAL AND METHODS

Synthesis and purification of colloidal AF^{14}CF

Sodium hexa[^{14}C]cyanoferrate (23 mCi/mmol; 851 MBq/mmol) with a radiochemical purity higher than 99% was obtained from Rahn, (Zurich, Switzerland) and stored before use at -20°C . Alkaline sodium [^{14}C]cyanide (55 mCi/mmol; 2.04 GBq/mmol) was obtained from Rahn, for the study of its adsorption on AFCF. Ammonium hexacyanoferrate was obtained from Loba Feinchemie (Fischamend, Austria) and ferric chloride from E. Merck (D-6100 Darmstadt, FRG). The synthesis of AF^{14}CF was performed according to the following reaction using 0.05 mmol sodium hexa[^{14}C]cyanoferrate as tracer with 24.64 mmol ammonium hexacyanoferrate:

The ratio Na/NH_4 was only 0.2% in the final product. Ammonium hexacyanoferrate 1 M (24.6 ml) was stirred continuously during drop-wise addition of an equimolar quantity of FeCl_3 3.5 M (7 ml). The product of the reaction was dialysed for 6 d in a carefully washed dialysis tubing of 31.7 mm o.d. and 1.5–2.0 nm pore diameter (Medicell International Ltd, London, UK) in order to remove ammonium chloride, unreacted labelled sodium hexacyanoferrate and impurities present in the starting

material. Distilled water used for dialysis was changed twice daily for the first 2 d and then only once daily. The transfer of labelled products was assessed quantitatively by counting samples of 0.5 and 1 ml dialysate with a liquid scintillation counter (Mark III, Searle and Instagel II Cocktail, Packard Instrument International SA, Zurich, Switzerland). The labelled products present in 500 ml dialysate were then concentrated to 10 ml under vacuum at 40 °C (Rotavapor, Büchi Laboratory Techniques Ltd, CH-9230 Flawil, Switzerland) and chromatographed on cellulose thin layer plates (Merck, 0.1 mm thickness) with either (i) methanol/water (95:5, v/v) or (ii) ethanol/pyridine/water/ammonia (60:20:16:4, v/v) as solvent systems. The localization and quantitative determination of labelled products after chromatography was performed with thin-layer chromatography (t.l.c.) Automatic Linear Analyzer (Berthold, Wildbad, FRG). Cyanide (Merck) and thiocyanate (Fluka AG, CH-9470 Buchs, Switzerland) detection on t.l.c. were obtained with 2', 7'-dichlorofluorescein spray reagent 0.1 % (Merck).

The adsorption of cyanide on AF¹⁴CF and the effect of pH on this adsorption was examined by adding ammonium [¹⁴C]cyanide (4.6 MBq, 0.1 mg) to 50 mg dry AF¹⁴CF; after mixing for 30 min at room temperature, 1 ml of 0.1 M-phosphate buffer (pH 4.4; 5.4; 6.0; 6.6; 7.5; 10.5) was added. One hour later, the suspension was counted before and after ultrafiltration (Amicon, Centriflo). In another experiment, the previously mentioned dialysis procedure was used after the addition of the ammonium [¹⁴C]cyanide to the unlabelled complex to study the release of radioactivity through the membrane.

In vitro experiments with rumen fluid

Rumen fluid was collected from a fistulated cow at the School of Veterinary Medicine, Large Animal Clinic, University of Berne, into a Dewar flask previously warmed with hot water (37 °C). CO₂ was passed into the flask to displace O₂ and to maintain anaerobic conditions. The incubation of AF¹⁴CF in rumen fluid was performed according to a method originally published by Senshu (1966) and applied as described by Haselbach (1983). A shaking water bath was used to maintain the temperature of the rumen juice at 39 °C, and to mix AF¹⁴CF (3.3 MBq/mmol) added at a dose of 0.44 MBq/flask. Gas produced from the rumen fluid was transferred to two successive traps. The first contained 1250 ml 10⁻² M-cobalt chloride to trap H ¹⁴CN (Johnson & Isom, 1985), the second contained 500 ml 12 % ethanolamine in methanol, with phenolphthalein as indicator, to trap ¹⁴CO₂.

In vivo experiments on cows

An experiment was performed during July, August and September 1986 in Mühldorf, South Bavaria, to study the decrease of ¹³⁷Cs and ¹³⁴Cs in milk produced from cows fed contaminated fodder and receiving different treatments of AF¹⁴CF prepared and purified as for AF¹⁴CF. Four cows fed trefoil pasture containing an average of 70 Bq/kg total caesium then received fodder collected in the above area with a measured mean total caesium content of 3090 Bq/kg. AF¹⁴CF was premixed with maize silage and concentrated feed to assure the complete intake of the 1.5 or 3 g/d.

Experiments with AF¹⁴CF were performed on two 3-year-old cows (Simmental and Simmental-Red Holstein) weighing 610 kg. They were fed corn silage (20 kg/d) and hay (10 kg/d) and had free access to water. On day 0 of the experiment, each cow received via the oesophagus and through a 54 cm long flexible metal tube

(Hauptner 86) two gelatine capsules (29 ml, Wirtschaftsgenossenschaft Deutscher Tierärzte AG, Hannover, FRG) delivered with a piston. The capsules were filled with cellulose (HBS, Serva Feinbiochemica GmbH, Heidelberg, FRG) which protected a smaller capsule containing the labelled AFCF. A total of 18.8 MBq and 20.1 MBq respectively was administered in < 1 min, and after the administration of the second capsule, swallowing was verified manually and by measuring the lack of contamination in the residual fodder given on the same day.

Blood samples were taken from the jugular vein, 10 min after administration and then every 30 min for 10 h. Plasma was separated from blood cells by centrifugation at 4 °C and 1000 g (Hermle ZK 364 centrifuge) for 15 min and, while plasma radioactivity was determined immediately, blood cells were frozen for later analysis.

Urine was collected quantitatively using a urinal. Faeces were collected directly into a box placed behind the hind legs, or from the rubber groundsheet placed under the cow. Every day, faeces were weighed, homogenized and three separate portions taken for further analysis.

Cows were milked after careful washing of the udder to prevent contamination by faeces. Milk was collected twice daily and weighed. Samples of all washings (1, 2 and 5 ml portions), together with milk (1, 2 and 5 ml), urine (1, 2 and 5 ml) and plasma (1 and 2 ml) were counted for 20 min in a liquid scintillation counter (Betamatic, Kontron, Switzerland), while faeces (50–300 mg) and blood cells (50–300 mg) were counted after combustion (Harvey Instrument Corp., Hillsdale, NJ, USA).

Each hour for the first 10 h and then once daily, expired gas was collected into a Douglas bag (Linde, Plastigas) using a mask over the muzzle of the cow. This bag was punctured with a needle through a septum and the gas was pumped out to trap and measure H^{14}CN (Johnson & Isom, 1985) and $^{14}\text{CO}_2$ (Bircher & Preisig, 1981) after humidification of the gas in ethanol/water (1:1 v/v).

Nine days after the administration of AF^{14}CF , the two cows were killed and the organs (spleen, lungs, liver, heart and skeletal muscle) known to accumulate cyanide in animals (Yacoub *et al.* 1974) were removed and frozen at $-70\text{ }^{\circ}\text{C}$ before combustion of samples for the analysis of radioactivity by liquid scintillation counting. The carcasses were burned. The determination of radioactivity on a weight basis was calculated by measuring, for a constant time of 20 min, different weights of sample.

The radioactivity was considered significantly different from the background when more than three times the s.d. was observed. The s.d. was calculated according to:

$$\left[\text{s.d.} = \frac{C_s}{T_s^2} + \frac{C_b}{T_b^2} \right]^{\frac{1}{2}},$$

where C_s = total counts for sample plus background in time T_s and C_b = total counts for background in time T_b (Comar, 1955).

At the end of the experiments faeces of the two cows were analysed for parasites at the Institute for Veterinary Pathology, Dept. of Parasitology, University of Berne, Switzerland.

Fig. 1. Radioactivity appearing in dialysate expressed in MBq/h, during 6 d dialysis of $\text{NH}_4\text{Fe}^{3+}[\text{Fe}^{2+}(^{14}\text{CN})_6]$. Arrows (▲) show changes of distilled water, and dialysed products were identified in samples A, B and C (▼) taken after 10 h, 3 d and 6 d.

RESULTS

Purification of AF^{14}CF

A rapid dialysis of radioactive products in the first 3 h was followed by a long slow release which could still be detected on d 6 (Fig. 1). The identification of the labelled products going through the membrane was performed by chromatography with standards showing the presence of hexacyanoferrate and cyanide for the first 10 h (Fig. 2a). After 3 d (Fig. 2b), AF^{14}CF appeared and increased to become quantitatively the most important after 6 d (Fig. 2c). When (Fig. 2d) no radiochemical impurity could be demonstrated ($< 0.5\%$) in the labelled AFCF by radiochromatography, this latter material was subsequently used for the cow experiments.

Labelled cyanide added to AFCF was shown to be partly volatilized when acid buffer was added but its binding was also demonstrated as shown in Fig. 3. Chromatography in solvent (i) showed that purified $[^{14}\text{C}]$ cyanide, in trace amounts, was adsorbed on AFCF and was not dissociated by the chromatographic conditions. It was also observed that after 6 d dialysis, 12% of added $[^{14}\text{C}]$ cyanide was still present in AFCF. In AF^{14}CF used for cow experiments, the presence of traces of soluble cyanide was detected when dialysates were applied on cellulose thin layer plates with solvent (i) (Fig. 2a, b and c) and also solvent (ii).

In vitro experiments with rumen fluid

In these two *in vitro* tests, performed for 8 and 16 h respectively, no radioactivity was detected as H^{14}CN and only 0.2% of the added radioactivity was recovered as $^{14}\text{CO}_2$. The complete recovery of radioactivity in the rumen juice would suggest the stability of AF^{14}CF . However, the identification of the labelled products in the

Fig. 2. Radiochromatograms obtained after cellulose thin layer chromatography with solvent system (i): see Methods for definitions. The origin (O) and the front (F) are indicated on each radiochromatogram as well as the standards. The R_F obtained on cellulose thin layer plates for the pure standards, with the solvent systems (i) and (ii) were respectively: ammonium ferric hexacyanoferrate (0; 0-unstable); 1, ammonium hexacyanoferrate, (0.45; 0.33); 2, ammonium cyanide, (0.52; 0.06) and 3, ammonium thiocyanate, (0.73; 0.65). The results shown correspond to the analyses of dialysates taken after 10 h (a), 3 d (b) and 6 d (c); dialysed product used for the experiment (d), rumen juice at the end of the *in vitro* study (e) and the urinary and faecal extracts of the *in vivo* study (f).

Fig. 3. Effect of pH on the adsorption of labelled cyanide to ammonium ferric hexacyanoferrate (AFCF). The results are expressed as the % of total cyanide radioactivity.

Fig. 4. Effect of ammonium ferric hexacyanoferrate (AFCF) administered to cows fed contaminated fodder, on total milk ^{137}Cs and ^{134}Cs radioactivity. The arrows indicate the beginning of the administration of contaminated fodder ($\nearrow$) or AFCF ($\downarrow$). For explanations see Results, reduction of milk contamination.

rumen juice showed that AF^{14}CF (81% of total radioactivity) was dissociated into ammonium hexa[^{14}C]cyanoferrate (8%) and ammonium thiocyanate (11%) (Fig. 2 e).

In vivo experiment on cows

Reduction of milk contamination

As shown in Fig. 4 the mean total caesium radioactivity in milk of three cows (A) increased from 20 Bq/l to a plateau value of 220–260 Bq/l 10 d after feeding contaminated fodder. An increase of radioactivity in milk was completely prevented

in cow B by the addition of 1.5 g AFCF twice daily starting 2 d before the administration of contaminated fodder. The administration of 2×3 g AFCF/d to two cows (C) fed contaminated fodder produced a significant decrease of milk radioactivity within 3 d. A second phase appeared 7 d after the initiation of the treatment characterized by a continuous but smaller decrease of radioactivity. These kinetics are in agreement with a previous report which showed that the decline of ^{137}Cs in milk of lactating dairy cows was multiexponential (Sansom, 1966). During the treatment, the administration of either 1.5 or 3 g AFCF twice daily did not seem to modify the decontamination process.

Metabolic experiment with AF¹⁴CF

At no time after administration could a significant increase in radioactivity over background be observed in the cobalt chloride solution used to trap expired hydrocyanic acid. Very low but significant radioactivity (< 900 Bq/mmol expired CO_2), was recovered in all samples collected up to 48 h.

In plasma, radioactivity appeared 2 h after the administration, reached a plateau up to 24 h and decreased to background value on d 2. In blood cells, radioactivity can be detected in the samples collected during the first 7 h after administration, but maximal activity only reached 9000 Bq/g over the background.

Radioactivity found in urine exhibited similar changes in the two cows, with highest specific activity (1000–3000 Bq/l) found in urine collected 24–48 h after administration. Urine radioactivity then decreased slowly up to 9 d, where the measurements were still significantly higher than the background (100 Bq/l).

From the weight of urine collected each day, total radioactivity excreted can be calculated as well as the percentage of the dose and the accumulated percentage of the dose (Table 1). The mean daily excretion of urine was 11 ± 3 and 16 ± 3 kg and only 0.47 and 0.19% respectively of the administered dose was recovered in urine over 9 d. The absence of volatile labelled metabolites was demonstrated by the radioactivity counting of urine samples before and after evaporation to dryness with the distillate as well as after reconstitution in water. After evaporation, about 30% of urine labelled metabolites were extracted with methanol and the extract was applied on cellulose t.l.c. The radiochromatogram Fig. 2(f) showed the presence of hexacyanoferrate (89%) as the main excretory product, while thiocyanate (11%) chromatography was modified by urinary constituents and appeared as a large spot.

The percentage of radioactivity found in milk is presented in Table 1. The level of radioactivity in milk (300 Bq/l) was lower when compared with urine. Radioactivity was only significant for the first 5 d. The mean daily milk production was 15.4 ± 0.4 and 21.3 ± 0.5 kg respectively for both cows. The final total recovery during 9 d was only 0.068 and 0.071% of the radioactivity administered.

The total radioactivity found in urine and milk amounted to 0.54 and 0.26% of the administered dose and constituted an estimate of total intestinal absorption, assuming the absence of significant enterohepatic recycling followed by faecal excretion of absorbed radioactivity.

The daily excretion of faeces was quite different in quantity for the two cows and amounted to 28 ± 3 and 43 ± 2 kg respectively. In spite of dilution of radioactivity in faeces, significant measurements, over the background, were found on d 5 (cow 2) and d 9 (cow 1) after administration with maximum values of 250–400 Bq/g. The detection limit corresponded to a percentage of the dose smaller than 0.1%. After

Table 1. Excretion of radioactivity* in urine, milk and faeces of two cows after administration of ^{14}C -labelled ammonium ferric hexacyanoferrate

Days	Cows	Urine			Milk			Faeces		
		Weight, kg	% dose	Accumulated % dose	Weight, kg	% dose	Accumulated % dose	Weight, kg	% dose	Accumulated % dose
0.5	1	6.3	0.03	0.03	7.6	0.008	0.008	11.9	0.4	0.4
	2	7.9	0.02	0.02	8.6	0.008	0.008	16.6	0.03	0.03
1	1	8.3	0.13	0.16	8.5	0.012	0.020	19.7	43.2	43.6
	2	11.6	0.07	0.09	13.1	0.017	0.025	28.4	41.2	41.2
2	1	11.9	0.17	0.33	14.6	0.019	0.039	30.8	38.2	81.8
	2	13.6	0.08	0.18	22.3	0.016	0.041	41.8	35.5	76.7
3	1	8.2	0.06	0.39	15.8	0.015 ^a	0.054	32.8	9.0	91.1
	2	13.0	0.03	0.12	20.4	0.013 ^a	0.054	44.6	12.0	88.7
4	1	15.4	0.03	0.42	15.8	0.014 ^a	0.068	32.4	3.3	94.4
	2	18.45	0.02 ^a	0.14	21.2	0.008 ^b	0.062	45.4	1.8	90.5
5	1	12.3	0.01 ^a	0.44	15.6	0.003 ^b	0.071	26.1	0.15	94.5
	2	15.5	0.01 ^a	0.15	21.6	0.005 ^b	0.068	46.7	0.43	90.9
6-9	1	45.8	<0.03 ^b	0.47	61	NS	0.071	104.3	0.4	95.0
	2	72.8	<0.03 ^b	0.19	85	NS	0.068	167.3	NS	90.9

* Radioactivity was determined by counting different vols (1, 2 and 5 ml) or weights of samples. For some samples, significant results were obtained with only 2 ml urine or milk (a) or 5 ml urine or milk (b) or were not significantly different from the background (NS).

thawing and rehomogenization of the first 3 d faecal samples, the total recoveries in faeces were 95 ± 5 and $91 \pm 7\%$ respectively of the administered dose of AF^{14}CF . Faecal homogenates in water with the highest specific activity allowed the identification of the unchanged administered product (52%) with also hexacyanoferrate (16%) and thiocyanate (32%) (Fig. 3f).

Very low but significant labelling was observed in pancreas, liver, heart, spleen and muscle. This radioactivity did not seem to be evenly distributed in the organ and was not greater than 6 Bq/kg over the background in any tissue.

Parasitic infection of faeces

Trichuris ovis was found in the faeces of one cow and *Eimeria bovis* in the faeces of the other.

DISCUSSION AND CONCLUSIONS

Following caesium and strontium contaminations observed from nuclear weapon tests performed in 1950–1960, *in vivo* studies were initiated to propose animal additives for the decontamination of food, and particularly of milk. The increased excretion of radiocaesium by modification of dietary intake of potassium and sodium (Mraz *et al.* 1957), other primary nutrients (Johnson *et al.* 1968; Snipes & Riedesel, 1969) and even stable caesium (Furchner & Richmond, 1962) was not very effective. The mixing of additives such as vermiculite (Hazzard *et al.* 1969) or bentonite (Van den Hoek, 1976) up to 10% of daily food intake was necessary to achieve an 80% reduction of caesium in milk.

Our present study has shown that continuous treatment with only 3 g AF₆CF starting 2–3 d before feeding the contaminated fodder was necessary for total prevention of the transfer of radiocaesium to milk. After previous caesium contamination, it took a longer time to decrease radioactivity in the milk, owing to the biological half-life of absorbed caesium. Such treatments with AF₆CF or other hexacyanoferrate complexes have been shown to prevent caesium contamination of meat (Nezel, 1970) and to increase caesium excretion in faeces (Giese *et al.* 1970). Our metabolic study is in agreement with these observations because the administration of AF^{14}CF to two cows showed, as expected, that faecal elimination was the main pathway so that more than 90% of the oral dose was recovered from faeces. It can be concluded that this complex was not absorbed as well as the degradation products formed in the rumen and the gastrointestinal tract. Hexacyanoferrate and thiocyanate, which constituted 48% of the faecal homogenate, may have been quantitatively overestimated when compared with AF^{14}CF which is insoluble and settles rapidly. Experiments performed in rats (Dvořák *et al.* 1971) where colloidal ferrihexacyanoferrate was labelled with ^{59}Fe showed that 7% of the product administered orally disintegrated in the intestine into Fe^{3+} and $[\text{Fe}(\text{CN})_6]^{4-}$ and that about 2% of the latter was absorbed. Our studies with colloidal AF^{14}CF confirm both *in vitro* and *in vivo* that the complex tested is also partly dissociated, to an extent which is difficult to estimate with a ^{14}C -labelled compound.

With the administration of doses of AF^{14}CF as high as 20 MBq, significant amounts of radioactivity were detected in urine and milk. Total intestinal absorption estimated from cumulative urine and milk radioactivity amounted to 0.5 and 0.2% of the dose for each cow. Although these percentages are remarkably low, it was possible to identify hexacyanoferrate and thiocyanate in urine. In agreement with Dvořák *et al.* (1971), a small fraction of hexacyanoferrate present in the intestine was

absorbed and, as shown after i.v. administration in animal and man, hexacyanoferrate was mainly excreted unchanged in urine (World Health Organization, 1974). However, a possible adverse effect linked to the presence of $[\text{Fe}(\text{CN})_6]^{4-}$ in the body was discussed by Bozorgzadéh & Catsch (1972). When potassium hexacyanoferrate was given parenterally, a pronounced delay of caesium excretion was shown, when compared with oral administration, explained by the formation of insoluble caesium complexes in the body which are unavailable for elimination. The authors concluded that the prolonged administration of these complexes resulted in two diametrically opposed modes of action—firstly, an enhancement of the elimination of caesium and, secondly, a superimposed retardation of the excretion rate, due mainly to caesium-complex retention by liver, spleen and skeleton. This observation has not yet been confirmed by other studies and is in disagreement with work showing that stored hexacyanoferrate observed in rabbits 13 d after repeated injection disappeared at 52 d (Gersch & Stieglitz, 1934). Moreover, a 90 d feeding study of 5% sodium hexacyanoferrate in the diet did not show tissue deposits (British Industrial Biological Research Association, 1969). In our study, the level of radioactivity detected in the organs studied was low after 9 d. The long term retention of caesium in animals fed contaminated diets and untreated or treated with AFCF or hexacyanoferrate is in progress. The low but significant labelling of blood cells and expired CO_2 is in agreement with cyanide formation and metabolism. Thiocyanate is the main detoxification product of cyanide and is present both in urine and milk. Because the radioactivity in milk was so low that it was impossible to identify the labelled products, and if we consider that the whole radioactivity found in milk was thiocyanate, a daily maximum thiocyanate secretion of 3 mg from 3 g AFCF can be calculated. The concentration of thiocyanate in milk will increase to a maximum value of 0.2 mg/l, corresponding to an increase of 3–6% thiocyanate above the level naturally present in milk.

Hexacyanoferrate pigment, approved as a colour additive for externally applied drugs and cosmetics, must conform to specifications, and in particular must contain no more than 10 ppm of water soluble cyanide (42 *Federal Register*, 38562 July 29, 1977; amended, 43 FR 6937, Feb. 17, 1978; 44 FR 28321, May 15, 1979). It has been shown that methods described for the detection of free cyanide in hexacyanoferrous and hexacyanoferric salts and in Prussian blue (Kruse & Thibault, 1973; Willekens & Van den Bulcke, 1979) are not always specific, reproducible and sensitive (Thieman *et al.* 1979) and particularly that cyanide spiked into the sample before the extraction step was adsorbed by the pigment.

This observation was confirmed in this work and it was also shown that free cyanide can still be adsorbed to AFCF after 6 d dialysis, while thin layer chromatography did not reveal its presence. It can thus be suggested that the radioactivity recovered as $^{14}\text{CO}_2$ in the *in vitro* experiment, and as thiocyanate in the urine of the cows was derived from cyanide adsorbed in the complex and released in the rumen.

In the case of cows treated with AFCF for the removal of ^{137}Cs from milk, it will be necessary to establish specifications for the content of soluble cyanide in the product and to conform to good manufacturing practice warranting such levels.

It was expected that the presence of intestinal parasites by provoking intestinal irritation could increase AFCF absorption. This assumption was ruled out when *Eimeria bovis* was identified in one cow without enhanced absorption of radioactivity during intestinal transit.

In conclusion, this study demonstrated that the absorption of ^{137}Cs present

in fodder and its secretion in milk can be effectively and safely prevented by using AFCE, which is easily manufactured at relatively low cost.

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