Bioavailability of Iron and Cyanide from ^{59}Fe- and ^{14}C-Labelled Hexacyanoferrates(II) in Rats

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"Soluble" ($KFe^{III}[Fe^{II}(CN)_6]$) and "insoluble Prussian blue" ($Fe^{III}_4[Fe^{II}(CN)_6]_3$ labelled with ^{59}Fe either in the ferric (Fe^{III}) or ferro (Fe^{II}) position and ^{14}C in the cyanide group were synthesized and administered intraperitoneally or orally to adult female rats with normal body iron stores.

Following *i.p. injection* of $KFe[Fe(CN)_6]$, the colloidal complex is disintegrated into ferric iron and hexacyanoferrate(II) anion almost completely. About 96% of the *ferric* iron was retained in the body. Nearly 90% of both ferrous iron and cyanide were excreted with the urine within 7 days after i.p. injection, indicating that most of the undissociated hexacyanoferrate(II) anion ($[Fe(CN)_6]^{4-}$) was excreted through the kidney. Only 9% of the *ferrous* iron from $[Fe(CN)_6]^{4-}$ was found mainly in carcass, liver and gut. As the ^{59}Fe/^{14}C-ratios in organs were found close to 1.0, the dissociation of the hexacyanoferrate(II) anion can only be small *in vivo*. No detectable $^{14}CO_2$-activity (< 0.01%) was monitored in the breath of rats after i.p. injection of the ^{14}C-labelled $KFe[Fe(CN)_6]$, also indicating that no significant amounts of cyanide were released after parenteral administration.

After *oral administration* of the soluble and insoluble Prussian blue, 0.3–0.7% of the ferric iron was absorbed and retained mainly in carcass, liver and blood. Only 0.06–0.18% of the ferrous iron was absorbed and mostly excreted with the urine (0.05–0.15%), so that only 0.01–0.03% of the oral ferrous ^{59}Fe was retained in the body after 7–10 days. Very small fractions of ^{14}C-label from the ^{14}CN-group of the soluble and insoluble hexacyanoferrate(II) were observed in the exhaled air (0.04–0.08% of the oral dose). From the $^{14}CO_2$-exhalation, the ^{14}C-urine excretion and the distribution of iron in blood and organs it can be concluded that the hexacyanoferrate(II) moiety disintegrated only to a small extent in the intestinal tract after oral administration. From a dose of 36 mg hexacyanoferrate(II)/kg, an amount of free (non-complex bound) cyanide can be calculated which is in maximum two orders of magnitude below the LD_{100}-level. Thus, the very low bioavailability of iron and cyanide from hexacyanoferrate(II) compounds after oral application is demonstrated in rats. In the case of a severe nuclear accident, appropriate doses of "soluble" and "insoluble" Prussian blue can be used as safe and effective antidote against radiocaesium contamination.

Introduction

Prussian blue derivatives ("soluble", $KFe^{III}[Fe^{II}(CN)_6]$; "insoluble", $Fe^{III}_4[Fe^{II}(CN)_6]_3$) adsorb caesium or thallium selectively even in sodium or potassium rich milieu [1–2]. The efficacy of hexacyanoferrate(II) compounds as scavenging agents for radiocaesium isotopes has been used for their separation from nuclear fission products and dilute solutions [3–4]. Furthermore, hexacyanoferrates(II) have been used for preventing the intestinal absorption [5–6] and the decorporation of already absorbed radiocaesium in animals [7–8] and man [9–10].

Following the nuclear reactor catastrophe in Chernobyl in April 1986, which resulted in an Europe wide contamination of the food chain with the potential hazardous radionuclides ^{134}Cs and ^{137}Cs, the efficacy of practical procedures for the prevention of radiocaesium incorporation from contaminated diets was reinvestigated and improved [11–14].

Empirically, no toxic side effects, affecting health and development of animals were observed after application of even large dosages of hexacyanoferrates(II), *i.e.* 2% $KFe[Fe(CN)_6]$ in drinking water for a 12 weeks period in rats [15] and 5–10 g $NH_4Fe[Fe(CN)_6]$ for 15 d in sheep [12]. From these studies, a complete non-absorption of soluble and insoluble Prussian blue in the intestinal tract after oral application was assumed and appropriate pharmaceutical preparations were

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authorized as antidotes against (radio)caesium and thallium intoxications for example in West-Germany (*i.e.* "Radiogardase-Cs", Heyl, Berlin, F.R.G.).

However, prior to the widespread use of hexacyanoferrates(II) in the case of Chernobyl-like severe nuclear accidents in the future, the limited bioavailability of iron and esp. of the CN^- group should be demonstrated also by experimental investigations. The absorption of ferrous and ferric iron from Prussian blue was shown to be small in rats [15] and piglets [14]. In the present study, various ^{59}Fe and ^{14}C labelled hexacyanoferrate(II) compounds with high purity were synthesized and administered orally or parenterally to rats. Because of the high specific activities used and the low body weight of rats as compared to cows or piglets, the absorption, distribution and excretion of even small amounts of ^{59}Fe and ^{14}C could be studied with more precision than in earlier studies.

Materials and Methods

All chemicals used were of analytical grade and obtained from E. Merck, Darmstadt. $K^{14}CN$ (37 MBq (1 mCi)/1.19 mg) and $^{59}FeCl_3$ (37 MBq (1 mCi)/184 µg) were obtained from Amersham International, England. $^{59}Fe(III)$ in acid solution was reduced to $^{59}Fe(II)$ by the addition of a 10-fold molar excess of ascorbic acid.

Synthesis of ^{14}C and ^{59}Fe labelled hexacyanoferrates(II)

$K_4[^{59}Fe(^{14}CN)_6] \cdot 3\ H_2O$. To a solution of 139 mg (0.5 mmol) $FeSO_4 \cdot 7\ H_2O$ in 20 ml of water were added 14.8 MBq (400 µCi) $^{59}Fe(II)$ in 0.05 M hydrochloric acid. After stirring under N_2-atmosphere for 15 min, a solution of 260 mg (4 mmol) potassium cyanide (37 MBq, 1 mCi ^{14}C) in 10 ml H_2O was slowly added. The alkaline reaction mixture was kept under reflux for 15 min. After cooling to room temperature, the mixture was filtered. The clear filtrate was acidified by the addition of 1 M HCl and evaporated to dryness under reduced pressure. The yellow residue was resolved in 5 ml of water and precipitated by adding an equal volume of ethanol. After centrifugation at 3000 g for 30 min, the supernatant was discharged and the pellet was lyophilized. Yield, 173 mg (82%); spec. activ., 68.8 kBq/mg (1.86 µCi/mg) ^{59}Fe, 95.1 kBq/mg (2.57 µCi/mg) ^{14}C; Fe, calc. 13.21%, found 13.26%.

$Fe_4[^{59}Fe(^{14}CN)_6]_3 \cdot 15\ H_2O$. To a solution of 100 mg (0.24 mmol) $K_4[^{59}Fe(^{14}CN)_6]$ in 100 ml of water was added a solution of 130 mg (0.48 mmol) $FeCl_3 \cdot 6\ H_2O$ in 20 ml 0.01 N HCl under stirring. The reaction mixture was kept at room temperature for 18 h. After centrifugation (5000 × *g*, 30 min) the pellet was washed 3 times with 0.1 M HCl and water. Yield, 72.4 mg (80.1%); spec. activity, 78.8 kBq/mg (2.13 µCi/mg) ^{59}Fe, 106.9 kBq/mg (2.89 µCi/mg) ^{14}C; Fe, calc. 34.62%, found 34.18%.

$^{59}Fe_4[Fe(^{14}CN)_6]_3 \cdot 15\ H_2O$. This compound was prepared by the addition of $^{59}FeCl_3$ to $K_4[Fe(^{14}CN)_6]$ as described above. Spec. activity, 90.7 kBq/mg (2.45 µCi/mg) ^{59}Fe, 199.8 kBq/mg (5.40 µCi/mg) ^{14}C; Fe calc. 34.62%, found 34.50%.

$KFe[^{59}Fe(^{14}CN)_6] \cdot 2\ H_2O$. To a solution of 139 mg (0.5 mmol, 600 µCi $^{59}Fe^{2+}$) $FeSO_4 \cdot 7\ H_2O$ was slowly added an aqueous solution of 326 mg (5 mmol, 1 mCi ^{14}C) potassium cyanide. After heating to 100 °C for 15 min the alkaline solution was cooled and filtered. The clear yellow filtrate was acidified by the addition of HCl. The yield of potassium hexacyanoferrate(II) formed was determined photometrically (aqueous solution, $\epsilon_{366} = 68.39$). An equimolar amount of $FeCl_3$ in 0.01 N HCl was added under stirring. After 2 h the deep blue colloidal solution was dialyzed 6 times against 3 l of water for 72 h. After evaporation to dryness under reduced pressure the residue was lyophilized. Yield 144 mg (84%); spec. activ. 126.2 kBq/mg (3.41 µCi/mg) ^{59}Fe, 92.5 kBq/mg (2.50 µCi/mg) ^{14}C. Fe, calc. 32.57%, found 32.14%.

$K^{59}Fe[Fe(^{14}CN)_6] \cdot 2\ H_2O$. This compound was prepared by the addition of $^{59}FeCl_3$ to $[Fe(^{14}CN)_6]^{4-}$ as described for $KFe[^{59}Fe(^{14}CN)_6] \cdot 2\ H_2O$. Spec. activ. 95.1 kBq/mg (2.57 µCi/mg) ^{59}Fe, 220.2 kBq/mg (5.95 µCi/mg) ^{14}C. Fe, calc. 32.57%, found 32.21%.

For determination of iron in the hexacyanoferrates(II), the solid samples were treated with boiling concentrated H_2SO_4. The iron sulfate formed was dissolved in 0.1 M HCl and iron was determined spectrophotometrically using bathophenanthrolin as color reagent.

Animal experiments and sample workup

Female Wistar rats (250–280 g; Wiga, Hannover, F.R.G.), kept on a standard food in pellet form (Altromin 1328; iron content, 250 mg/kg; Altromin, Lage, F.R.G.) and tap water ad libitum were used. Due to the high iron content of the diet, the liver iron concentration was slightly increased (0.4–0.5 mg Fe/g liver wet weight). The rats were fasted 24 h before and 4 h after the respective administration of labelled hexacyanoferrates(II). Aqueous solutions or suspensions of HCF (10–13 mg, 0.96–1.70 MBq (26–46 μCi) ^{59}Fe and 1.22–2.89 MBq (33–78 μCi) ^{14}C) were administered by intraperitoneal injection or gastric intubation. After administration of hexacyanoferrates(II) the rats were kept in individual metabolic cages over 7 days for a quantitative collection of urine and faeces.

The whole body retention of injected or absorbed oral ^{59}Fe was measured 7 days after application in the center of a 200 cm long 4 π-geometry whole body radioactivity detector with liquid organic scintillator in the energy range from 980–3000 keV as described for humans [16]. The expired air of the rats was measured for $^{14}CO_2$-radioactivity during the first 24 h after i.p. and oral application of ^{14}C-labelled hexacyanoferrates(II) in a modified $^{14}CO_2$ respiration analyzer type FHT 50 A (Frieseke and Höpfner, Erlangen, F.R.G.). The detection limit was 0.01% of the administered ^{14}C-activity. Within each experiment, the noise level of the system was controlled for exterior radiation effects (^{59}Fe in rats) by disconnecting the air flow between the metabolic cage and the $^{14}CO_2$-flow through methane proportional detector for a 60 min period (Fig. 1).

The rats were sacrificed by exsanguination from the abdominal aorta while under light ether narcosis. The theoretical blood volume was calculated as 5.6% of the body weight, the specific weight of blood as 1.054 g/ml [17]. Blood, liver, spleen, kidneys, heart/lung and gut were removed and the ^{59}Fe-content of organs and carcass measured within the whole body radioactivity detector. For ^{14}C-estimation, organs were dried by lyophilization and portions of 0.5 g each converted to $^{14}CO_2$ using a sample oxidizer (Tri-Carb model 306, Carbosorb CO_2-absorber, Packard-Instrument Co.). The ^{14}C-activity was measured by liquid scintillation counting using a permafluor premix cocktail and a TriCarb Model 4430 liquid scintillation spectrometer.

Results

"Soluble" ($KFe[Fe(CN)_6$) and "insoluble" ($Fe_4[Fe(CN)_6]_3$) "Prussian Blue" labelled with ^{59}Fe in the ferric or ferrous position and ^{14}C in the cyanide group were synthesized and used for the estimation of their bioavailability in rats. $^{59}FeCl_3$ and $K_4[^{59}Fe(^{14}CN)_6]$ were used as reference compounds.

Bioavailability of ferric iron from hexacyanoferrates(II)

Ferric chloride. After *oral administration* of a tracer dose of [^{59}Fe]ferric chloride, about 8% of the dose was absorbed. This iron was incorporated into the hemoglobin of circulating erythrocytes or retained in carcass and liver, whereas the urine excretion was negligible (Tables I and II).

$K^{59}Fe[Fe(^{14}CN)_6]$. Following the *i.p. injection* of a 10 mg amount (1.6 mg ferric iron), the ferric iron was almost completely retained after 7 days (96.2% whole-body-retention, Table I). The organ distribution is similar to that found after oral administration of $^{59}FeCl_3$, except that a large fraction (38%) of the *ferric* iron was associated with the gut (Table II). On the first look, this might indicate that a significant portion of the colloid-soluble hexacyanoferrate(II) complex had reacted with the tissue, became insoluble and metabolically inert. However, considering the high urine excretion of ^{14}C from this experiment and the respective ^{59}-Fe- and ^{14}C-data from the analogous experiment using $KFe[^{59}Fe(^{14}CN)_6]$, this non-metabolized fraction of $KFe[Fe(CN)_6]$ is much smaller (about 10–15%).

The *oral administration* of a single or five subsequent daily doses of $K^{59}Fe[Fe(^{14}CN)_6]$ (10 mg each, 1.6 mg ferric iron), resulted in a ferric iron absorption of $0.7 \pm 0.3\%$ ($\equiv$ ^{59}Fe–WBR). The distribution of iron in blood and various organs was found to be similar to that of Fe(III) from ferric chloride (Table II).

$^{59}Fe_4[Fe(^{14}CN)_6]_3$. The absorption and organ distribution of labelled ferric iron was similar to that of Fe(III) from $K^{59}Fe[Fe(^{14}CN)_6]$ and $^{59}FeCl_3$ (Tables I and II).

Table I. Whole-body-retention (WBR) and erythrocyte incorporation of ^{59}Fe, faecal and urinary excretion of ^{59}Fe and ^{14}C (mean ± SD) 7–10 days after i.p. injection or oral application of $^{59}FeCl_3$ (tracer dose) and different labelled hexacyanoferrates(II) (10 mg) in groups of 2–5 rats.

Compound	*n*	^{59}Fe 7d-faeces (% of dose)	Absorption (100%-faec.)	7d-urine (% of dose)	WBR (% of dose)	Recovery (% of dose)	EI[1] (% WBR)	EI[2] (% of dose)	^{14}C 7d-urine (% of dose)
a) i.p. injection									
$K^{59}Fe[Fe(^{14}CN)_6]$	3	1.1±0.2	98.9	0.09 ±0.02	96.2 ±3.8	97.4±3.7	16.1± 2.2	15.3 ±2.0	84.0 ±2.5
$K_4[^{59}Fe(^{14}CN)_6]$	3	3.8±2.6	96.2	98.8 ±1.8	1.2 ±0.3	103.8±1.2	0.9± 0.2	0.0095±0.0012	99.4 ±4.1
$KFe[^{59}Fe(^{14}CN)_6]$	3	3.2±1.0	96.8	87.2 ±7.4	8.6 ±5.3	99.0±3.4	1.1± 0.1	0.089 ±0.043	88.5 ±4.5
b) oral administr.									
$^{59}FeCl_3$	2	93.1	6.9	0.007	7.9	101.0	69.0	4.7	–
$K^{59}Fe[Fe(^{14}CN)_6]$	5	101.1±3.7	–1.10	0.04 ±0.02	0.70±0.34	101.8±3.9	52.7±11.7	0.40 ±0.23	0.49±0.15
$K^{59}Fe[Fe(^{14}CN)_6]$ 5 applications[3]	2	97.2	2.8	0.02	0.31	97.5	66.1	0.21	0.45±0.07
$^{59}Fe_4[Fe(^{14}CN)_6]_3$	3	100.0±2.9	0	0.09 ±0.01	0.35±0.10	100.4±2.9	44.1±12.1	0.20 ±0.05	0.51±0.16
$K_4[^{59}Fe(^{14}CN)_6]$	3	94.4±2.9	5.6	2.5 ±0.8	0.09±0.02	97.0±2.1	6.7± 2.2	0.005 ±0.0007	2.8 ±0.5
$KFe[^{59}Fe(^{14}CN)_6]$	4	101.8±2.7	–1.8	0.15 ±0.06	0.03±0.01	101.9±2.8	37.9±18.2	0.013 ±0.001	0.33±0.03
$Fe_4[^{59}Fe(^{14}CN)_6]_3$	3	102.3±0.5	–2.3	0.05 ±0.03	0.01±0.002	102.3±0.6	34.8± 4.1	0.002 ±0.0009	0.21±0.03

[1] Erythrocyte incorporation ((^{59}Fe total blood activity/^{59}Fe-whole-body-retention) · 100) (theoretical total blood volume calculated as 5.6% of body weight).
[2] Erythrocyte incorporation ((^{59}Fe total blood activity/applicated ^{59}Fe-activity) · 100).
[3] Oral applications (10 mg each) on 5 subsequent days.

Table II. Distribution of ^{59}Fe-activity (in % of ^{59}Fe-whole-body-retention; mean ± SD) in organs of rats (n = 2–5), 7–10 days after i.p. injection or oral administration of $^{59}FeCl_3$ (tracer dose) and ^{59}Fe and ^{14}C labelled hexacyanoferrates(II) (10 mg).

Compound	Blood[1] [%]	Liver [%]	Spleen [%]	Kidney [%]	Heart + lung [%]	Gut [%]	Carcass [%]	Recovery [%]
a) i.p. injection								
$K^{59}Fe[Fe(^{14}CN)_6]$	10.5 ±1.1	22.7± 1.8	1.5±0.7	0.38± 0.05	0.74±0.32	37.5±8.8	26.8± 6.1	95.6± 7.7
$K_4[^{59}Fe(^{14}CN)_6]$	0.46±0.1	8.1± 0.1	1.6±0.1	16.3 ± 1.4	1.2 ±0.2	7.0±1.3	65.9± 2.3	100.1± 0.10
$KFe[^{59}Fe(^{14}CN)_6]$	1.31±0.9	36.6±10.7	2.4±0.9	7.7 ± 3.3	0.98±0.28	16.0±8.6	30.6± 4.7	100.5± 0.45
b) Oral administr.								
$^{59}FeCl_3$	31.9	23.2	1.9	1.8	2.3	3.9	31.7	96.7
$K^{59}Fe[Fe(^{14}CN)_6]$	25.6 ±6.5	27.2± 8.3	1.7±0.4	1.5 ± 0.3	1.9 ±0.4	4.8±1.2	34.4±14.0	97.2± 9.7
$K^{59}Fe[Fe(^{14}CN)_6]$ 5 applications[2]	34.0	20.7	1.5	1.5	2.4	5.3	28.1	93.4
$^{59}Fe_4[Fe(^{14}CN)_6]_3$	30.0 ±8.0	21.2± 4.8	1.2±0.4	1.4 ± 0.2	1.8 ±0.8	4.5±0.3	51.2±10.6	107.8± 4.4
$K_4[^{59}Fe(^{14}CN)_6]$	2.8 ±1.0	9.9± 3.6	1.1±0.3	24.2 ±14.8	1.1 ±0.5	11.2±0.6	53.6±11.0	103.8± 7.9
$KFe[^{59}Fe(^{14}CN)_6]$	24.9 ±3.4	25.9± 2.6	1.7±0.2	8.2 ± 2.1	4.6 ±1.4	5.8±3.5	28.6± 6.7	99.7± 2.5
$Fe_4[^{59}Fe(^{14}CN)_6]_3$	18.5 ±4.1	21.5± 2.3	1.3±0.2	8.4 ± 1.8	2.5 ±1.3	5.4±1.4	28.5± 9.1	86.1±11.6

[1] ^{59}Fe-activity in 6–10 ml of blood, recovered from punction of the aorta abdominalis (incomplete whole body blood volume; ≈ 14.8 ml theoretical blood volume for 265 g body weight).
[2] Oral applications (10 mg each) on 5 subsequent days.

Bioavailability of ferrous iron from hexacyanoferrates(II)

$K_4[^{59}Fe(^{14}CN)_6]$. Potassium hexacyanoferrate(II) (10 mg) was taken as reference for the bioavailability of hexacyanoferrate(II) anion.

Following *parenteral administration*, the ^{59}Fe-whole body retention from potassium hexacyanoferrate(II) was only small and 99% of the ^{59}Fe and ^{14}C dose were found in the urine within 7 days (Table I). The distribution of the retained ^{59}Fe-labelled ferrous iron in blood and organs was found

markedly different from oral ferric iron (Table II). Most of ^{59}Fe-label was found in carcass and kidneys, and only small amounts of the ferrous iron in liver and blood (Table II). After *oral administration,* 2.6% of ^{59}Fe(II) were absorbed, but again, only a minor fraction of absorbed iron was retained in the whole body (0.09% of dose, Table I). The organ distribution of ^{59}Fe was very similar to that found after i.p. injection, except showing more ^{59}Fe-activity in the blood after oral application.

$KFe[^{59}Fe(^{14}CN)_6]$. The *i.p. injection* resulted in a 8.6% ^{59}Fe-whole body retention after 7 days, whereas 87.2% of the ferrous iron and 88.5% ^{14}C were found in the urine (Table I). Similar to $K_4[^{59}Fe(^{14}CN)_6]$, the ^{59}Fe-activity in blood was very low, and the retained ^{59}Fe was found mainly in the carcass, liver, kidney and gut (Table II).

After *oral administration,* the whole-body-retention of ferrous iron was extremely low (0.03% of the dose). As indicated by the erythrocyte incorporation (Table I), more iron was found in the blood as compared to i.p. application (EI in % of WBR = 38% vs. 1.1%).

$Fe_4[^{59}Fe(^{14}CN)_6]_3$. Again, the absorption and retention of labelled ferrous iron was extremely small (0.01%) and similar to that of Fe(II) from $KFe[^{59}Fe(CN)_6]$ (Tables I and II).

Bioavailability of cyanide from ^{14}C-labelled hexacyanoferrates(II)

The ^{14}C-activities found in rat organs were small in general (< 1% of dose), but much higher after parenteral administration of [^{14}C]hexacyanoferrates(II) as compared to oral administration (< 0.01%; Table III).

Table III. ^{59}Fe- and ^{14}C-activity in organs of rats (n = 2–5), 7–10 days after i.p. injection and oral administration of $^{59}FeCl_3$ (tracer dose) and ^{59}Fe and ^{14}C labelled hexacyanoferrates(II) (10 mg) (in % of administered dose).

Compound	Rats n	Blood ^{59}Fe	^{14}C	Liver ^{59}Fe	^{14}C	Spleen ^{59}Fe	^{14}C	Kidney ^{59}Fe	^{14}C	Heart + lung ^{59}Fe	^{14}C
a) i.p. injection											
$K^{59}Fe[Fe(^{14}CN)_6]$	3	9.86 ±1.04	0.023 ±0.010	21.4 ±1.77	0.750 ±0.046	1.41 ±0.601	0.068 ±0.020	0.407 ±0.045	0.307 ±0.064	0.700 ±0.305	0.035 ±0.007
$K_4[^{59}Fe(^{14}CN)_6]$	3	0.005 ±0.0001	0.003 ±0.001	0.097±0.022	0.093 ±0.024	0.019 ±0.003	0.019 ±0.004	0.198 ±0.058	0.200 ±0.064	0.015 ±0.001	0.015 ±0.001
$KFe[^{59}Fe(^{14}CN)_6]$	3	0.033 ±0.003	0.006 ±0.002	0.930±0.709	0.691 ±0.553	0.050 ±0.021	0.034 ±0.011	0.288 ±0.124	0.245 ±0.110	0.026 ±0.010	0.017 ±0.008
b) oral administr.											
$^{59}FeCl_3$	2	2.52		1.82		0.153		0.140		0.182	
$K^{59}Fe[Fe(^{14}CN)_6]$	5	0.196 ±0.121	0.004 ±0.0008	0.209±0.12	0.003 ±0.000	0.013 ±0.006	0.0003 ±0.0008	0.012 ±0.005	0.003 ±0.001	0.013 ±0.006	0.0008 ±0.0002
$K^{59}Fe[Fe(^{14}CN)_6]$	2	0.108	0.017	0.064	0.018	0.005	0.0001	0.0047	0.0033	0.008	0.0005
5 applications											
$^{59}Fe_4[Fe(^{14}CN)_6]_3$	3	0.135 ±0.035	0.005 ±0.001	0.098±0.031	0.003 ±0.001	0.005 ±0.002	0.0003 ±0.0001	0.0064±0.013	0.004 ±0.003	0.008 ±0.004	0.0009 ±0.0003
$K_4[^{59}Fe(^{14}CN)_6]$	3	0.003 ±0.0009	0.001 ±0.0004	0.009±0.002	0.009 ±0.001	0.001 ±0.0003	0.0009 ±0.0005	0.002 ±0.007	0.019 ±0.004	0.0009±0.0002	0.001 ±0.0005
$KFe[^{59}Fe(^{14}CN)_6]$	4	0.008 ±0.001	0.003 ±0.001	0.008±0.003	0.003 ±0.0005	0.0005 ±0.0001	0.0002 ±0.0001	0.003 ±0.0003	0.003 ±0.0004	0.003 ±0.0007	0.0007 ±0.0003
$Fe_4[^{59}Fe(^{14}CN)_6]_3$	3	0.0013±0.0006	0.0002±0.0001	0.002±0.0004	0.0002±0.00004	0.00009±0.00004	0.00002±0.00001	0.0006±0.0002	0.0011±0.0006	0.0002±0.0001	0.00006±0.00001

Table IV. ^{59}Fe/^{14}C-ratio (mean ± SD) in blood and organs of rats (n = 3–5) 7–10 days after administration of 10 mg of ^{59}Fe and ^{14}C labelled hexacyanoferrates(II).

Compound	Blood	Liver	Spleen	Kidney	Heart + lung
a) i.p. injection					
$K^{59}Fe[Fe(^{14}CN)_6]$	471 ±201	29 ± 3.8	21 ± 6.4	1.4±0.2	20 ±4.5
$K_4[^{59}Fe(^{14}CN)_6]$	1.9± 0.9	1.0± 0.06	1.0±0	1.0±0	1.0±0
$KFe[^{59}Fe(^{14}CN)_6]$	6.0± 1.2	1.4± 0.06	1.5±0.2	1.2±0.06	1.7±0.3
b) Oral application					
$K^{59}Fe[Fe(^{14}CN)_6]$	66 ± 31	85 ±27	53 ±29	3.6±2.2	20 ±7.5
$K^{59}Fe[Fe(^{14}CN)_6]$	67 ± 9.2	37 ± 4.2	46 ± 3.5	1.5±0.2	17 ±2.8
5 applications					
$^{59}Fe_4[Fe(^{14}CN)_6]_3$	26 ± 6.0	35 ± 7.2	20 ± 6.0	2.6±2.2	8.5±2.7
$K_4[^{59}Fe(^{14}CN)_6]$	2.5± 1.4	1.0± 0.1	1.4± 0.7	1.1±0.4	0.8±0.2
$KFe[^{59}Fe(^{14}CN)_6]$	3.3± 0.9	3.0± 0.6	3.4± 1.6	1.0±0.05	2.4±1.7
$Fe_4[^{59}Fe(^{14}CN)_6]_3$	8.4± 4.0	6.1± 2.6	6.1± 5.3	0.6±0.5	3.4±2.4

With respect to the expired $^{14}CO_2$-activity there was found a difference between parenteral and oral application of [^{14}C]hexacyanoferrates(II) (Table V). Following i.p. injection, the signals measured were within the noise level (< 0.01% of dose), whereas after oral application, a small but significant fraction of expired $^{14}CO_2$ (≈ 0.1% of dose) was measured. A typical $^{14}CO_2$-expiration profile from a rat given $K^{59}Fe[Fe(^{14}CN)]$ in aqueous solution through a gastric tube is shown in Fig. 1, 0.06% of the ^{14}C-activity of $^{14}CO_2$ were detected in the breath within 24 h after application.

Table V. Amount of expired $^{14}CO_2$ (% of administered dose; mean ± SD) during 24 h after oral application of 10 mg amounts of different ^{14}C-labelled hexacyanoferrates(II) in rats (n = 3–5).

Compounds	Expired $^{14}CO_2$
a) i.p. injection	
$K^{59}Fe[Fe(^{14}CN)_6]$	< 0.01
$K_4[^{59}Fe(^{14}CN)_6]$	< 0.01
$KFe[^{59}Fe(^{14}CN)_6]$	< 0.01
b) Oral application	
$K^{59}Fe[Fe(^{14}CN)_6]$	0.08±0.01
$^{59}Fe_4[Fe(^{14}CN)_6]_3$	0.08±0.01
$K_4[^{59}Fe(^{14}CN)_6]$	0.04±0.01
$KFe[^{59}Fe(^{14}CN)_6]$	0.08±0.01
$Fe_4[^{59}Fe(^{14}CN)_6]_3$	0.08±0.01

Discussion

The efficacy of iron(III) hexacyanoferrate(II) for the prevention of intestinal radiocaesium uptake or decorporation in animals and humans is well established [5, 7, 9]. However, up to now, very limited information concerning the bioavailability of iron and cyanide from these compounds is available.

Regarding the bioavailability of iron and cyanide after oral administration of hexacyanoferrates(II), the dissociation and the purity of the respective preparation have to be considered:

a) In the aqueous milieu of the intestinal tract the dissociation of hexacyanoferrates(II) can be described by a two step reaction:

$$KFe[Fe(CN)_6] \rightleftharpoons Fe^{3+} + K^+ + [Fe(CN)_6]^{4-}$$
$$Fe_4[Fe(CN)_6]_3 \rightleftharpoons 4\ Fe^{3+} + 3\ [Fe(CN)_6]^{4-} \quad (1)$$

$$[Fe(CN)_6]^{4-} \rightleftharpoons Fe^{2+} + 6\ CN^- \quad (2)$$

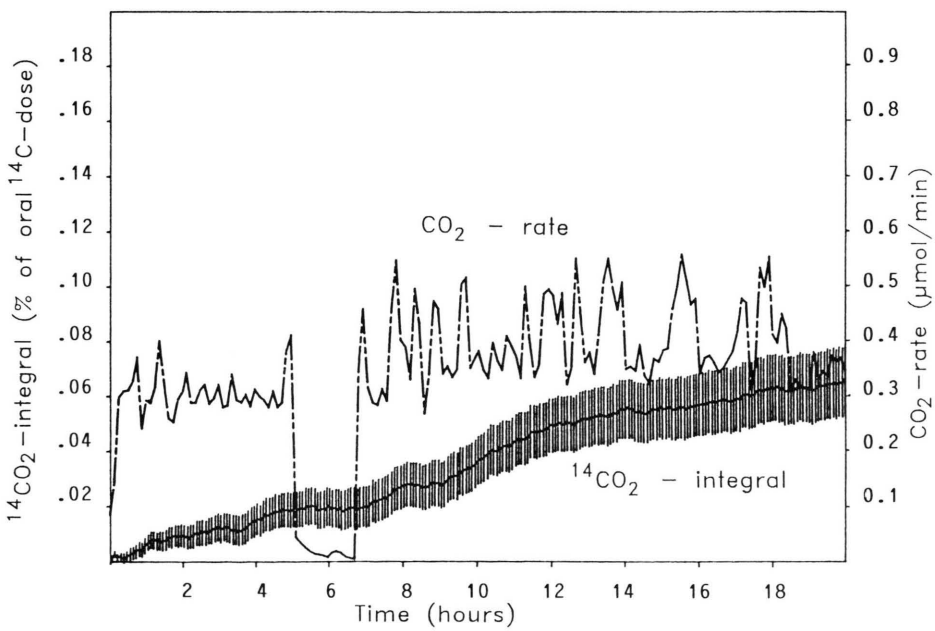

Fig. 1. 24 h-$^{14}CO_2$-exhalation profile after oral administration of an aqueous solution of 10 mg $K^{59}Fe[Fe(^{14}CN)_6]$ (1.0 ml; 22.9 µCi ^{59}Fe, 69.9 µCi ^{14}C) through a gastric tube to a rat. The $^{14}CO_2$-rate was monitored in a respiration analyzer. The noise level of the system (probably influenced by the administered ^{59}Fe-activity) was demonstrated by disconnecting the tube between analyzer and animal cage for 60 min while measuring (note the absent CO_2-rate and no further increase in exhaled $^{14}CO_2$ in this time interval, 5.5–6.5 h).

As a consequence of reaction 1 and 2, ferric and ferrous iron as well as cyanide are released from the finely dispersed hexacyanoferrate(II) compounds *prior to intestinal absorption.* From the chemistry of transition metal hexacyanoferates(II) it is known that, despite low pH values, these complexes are rather stable in aqueous solution ($K_{d,reac.2} = 10^{-35}$ mol/l, [18]). However, after oral application the influence of gastric and duodenal juice, bile and colon bacteria have to be considered, which may result in the release of significant amounts of iron and cyanide.

b) The hexacyanoferrate(II) anion ($[Fe(CN)_6]^{4-}$), as formed in reaction 1, can be hydrolyzed to ferrous iron and cyanide according to reaction 2 *before* and *after* intestinal absorption.

c) Crude (non-dialyzed) preparations of colloidal hexacyanoferrate(II) may contain large amounts of by-products ($[Fe(CN)_6]^{4-}$, $FeCl_3$, KCl etc.) which may serve in part as the real origin of absorbed iron and cyanide. In addition, significant amounts of anions (*e.g.* cyanide) can be adsorbed on the colloidal hexacyanoferrate(II) complex during preparation and it was suggested, that most of the bioavailable cyanide may be derived from this adsorbed cyanide fraction [19].

Metabolism of iron and cyanide in rats after intraperitoneal application of hexacyanoferrates(II)

From the experiment with i.p. injection of $K^{59}Fe[Fe(^{14}CN)_6]$ and $KFe[^{59}Fe(^{14}CN)_6]$ an almost complete *in vivo* dissociation of the colloidal hexacyanoferrate(II) complex according to reaction (1) can be concluded, since the urine excretion of cyanide as well as ferrous iron (probably non-dissociated $[Fe(CN)_6]^{4-}$) was 84–88% and the ferric iron was retained in the body almost quantitatively. This is in good agreement with earlier results from Dvorak *et al.* [15], who calculated an *in vivo* dissociation of the complex of about 60%. Concerning the stability of these compounds over a wide pH-range *in vitro,* this result may surprise. However, the equilibrium (reaction (1)) is probably shifted to the right by the reaction of apotransferrin with Fe^{3+} and the fast excretion of $[Fe(CN)_6]^{4-}$ through the kidney.

The *ferric* iron was almost completely retained in the body (probably used for haemoprotein biosynthesis and incorporated into the body iron stores). For labelled ferric iron, the $^{59}Fe/^{14}C$-ratio in rat organs (Table IV) was much higher than 1.0 (about 20 in liver and spleen and about 400 in blood), indicating that the ferric iron was mainly accumulated in the blood.

About 90% of the ^{59}Fe and ^{14}C from the i.p. injected $KFe[^{59}Fe(^{14}CN)_6]$ were excreted with the urine within 7 days. An additional fraction (3% ^{59}Fe) was found in the faeces (bile excretion ?).The $^{59}Fe/^{14}C$-ratios of rat organs were close to 1.0 (Table IV), indicating that the retained ferrous iron is distributed together with cyanide, probably as non-dissociated $[Fe(CN)_6]^{4-}$. Free ferrous iron, as product of reaction (2), is not stable under *in vivo* condition. After oxidation, most of this iron should be bound to apotransferrin and used in the erythropoesis or stored in ferritin. The $^{59}Fe/^{14}C$-ratio (between 1.0 and 1.7 in organs and 1.9–6.0 in blood) demonstrates that after i.p. administration of $K_4[^{59}Fe(CN)_6]$ and $KFe[^{59}Fe(CN)_6]$ respectively (Table III), only small fractions of the $[Fe(CN)_6]^{4-}$ anion are dissociated *in vivo* according to reaction (2). This can also be concluded from the results, that no $^{14}CO_2$ was detectable in the expired air of rats, receiving ^{14}C-labelled soluble Prussian blue by i.p. injection.

Absorption and metabolism of iron and cyanide in rats after oral application of hexacyanoferrates(II)

The very low bioavailability of the *ferric* iron (0.3–0.7% WBR, Table I) from "soluble" and "insoluble" Prussian blue corresponds to the well known poor iron absorption of trivalent iron containing inorganic and organic iron complexes in man [20], although rats are capable of synthesizing ascorbic acid and to reduce non available trivalent to absorbable bivalent iron at least to a certain degree.

About 2.6% of the *ferrous* iron from $K_4[Fe(CN)_6]$ (10 mg dose) were absorbed (whole-body-retention and urine activity), most of it (2.5%) excreted by the kidneys (Table I). This is in full agreement with results of Dvorak *et al.* [15], who described an absorption (urine excretion and organ activities) of 2.1%, independent of the oral dose (0.5–50 mg $K_4[Fe(CN)_6]$). The even lower bioavailability of the *ferrous* iron from both ferric hexacyanoferrates(II) (0.01–0.03% WBR, Table I) may be explained by practically no release

of absorbable ferrous iron from the hexacyanoferrate(II) anion within the upper gastrointestinal tract and the body.

Considering a ^{59}Fe absorption of 2.6% for the $[^{59}Fe(CN)_6]^{4-}$-anion, 0.18% for $KFe[^{59}Fe(CN)_6]$ and 0.06% for $Fe_4[^{59}Fe(CN)_6]$ (Table I) as a basis for estimation, an intestinal disintegration of 6.9% for $KFe[Fe(CN)_6]$ and 2.3% for $Fe_4[Fe(CN)_6]$ according to reaction (1) (see above) can be calculated (Fig. 2).

The erythrocyte incorporation (Table I) from the experiment with $KFe[^{59}Fe(^{14}CN)_6]$ and $K_4[^{59}Fe(^{14}CN)_6]$ are significantly higher after oral administration as compared to the experiment with i.p. injection. This difference must be interpreted as intestinal hydrolysis reaction of $[Fe(CN)_6]^{4-}$ according to reaction (2), followed by the independent absorption of ferrous iron and cyanide. The absorption of the small fraction of free cyanide account for the detectable $^{14}CO_2$-activity in the expired air of rats after oral administration of ^{14}C-labelled hexacyanoferrates(II).

Bioavailability of cyanide from oral hexacyanoferrates(II)

Regarding the high cyanide content of hexacyanoferrate(II) compounds, the potential toxicity of oral hexacyanoferrates(II) should mainly depend on the amount of non-complex-bound "free" cyanide in the body (see reaction (2)). Very small but still measurable amounts of cyanide and iron are absorbed and metabolized in rats from different hexacyanoferrates(II) after oral administration (Table I, Table III). From a 10 mg dose only about 0.04–0.08% of the ^{14}C-activity were exhaled as $^{14}CO_2$ within 24 h after application (Table V, Fig. 1). In the urine, collected for 7 days after oral application of [^{59}Fe]ferrous iron and ^{14}C-labelled hexacyanoferrates(II), the ^{14}C-activity was significantly higher than the ^{59}Fe-activity, indicating that

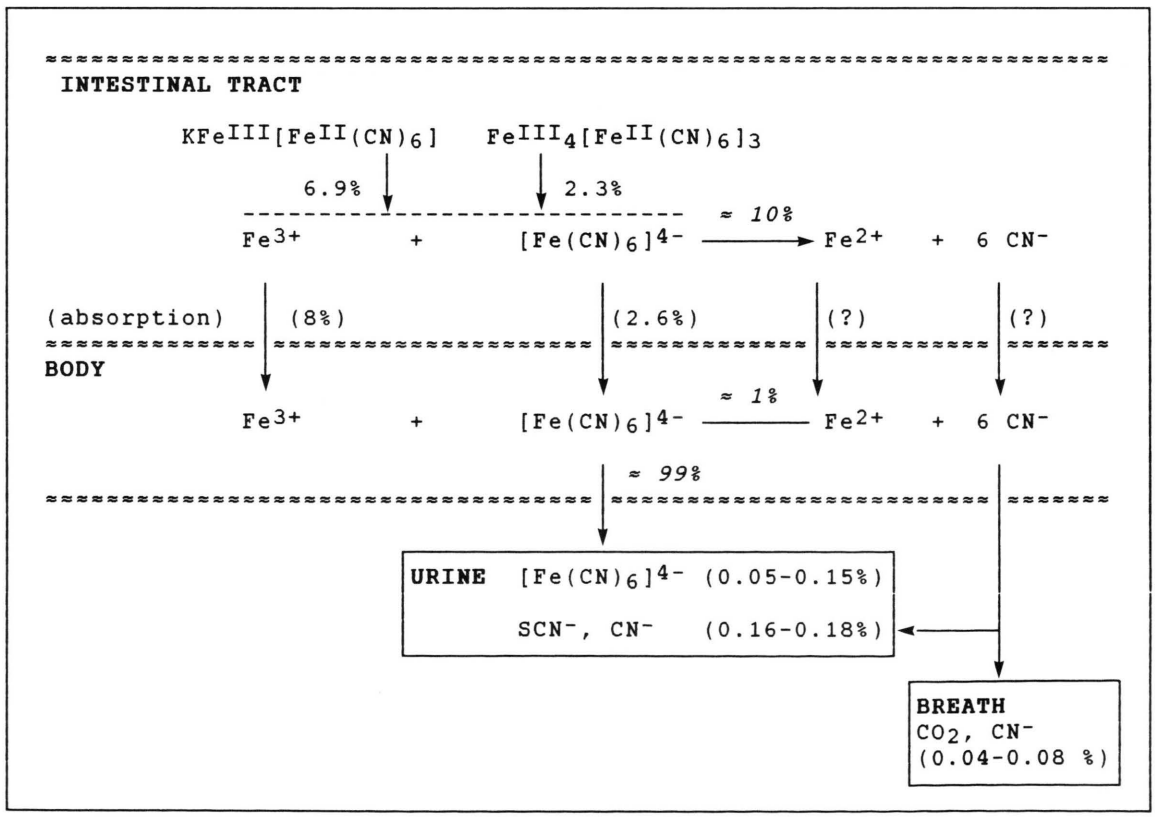

Fig. 2. Intestinal dissociation, absorption and metabolism of iron and cyanide from oral (36 mg/kg = 100%) hexacyanoferrates(II) in the rat.

Table VI. Amount of absorbed, non-complex-bound ("free") cyanide from different oral hexacyanoferrates(II) (10 mg) in rats (280 g) as calculated from the expired $^{14}CO_2$ activity and the excreted "free" ^{14}C-activity in the urine.

Compounds	Theor. cyanide content (in oral dose) [µg]	Cyanide equiv.[1] to expired $^{14}CO_2$ [µg]	"Free" cyanide[2] in urine [µg]	Cyanide absorbed[3] (calc. from exhalation) [µg/kg]	Cyanide absorbed[4] (calc. from urine excretion) [µg/kg]
$K^{59}Fe[Fe(^{14}CN)_6]$	4549	3.6	–	39	–
$^{59}Fe_4[Fe(^{14}CN)_6]_3$	4144	3.3	–	35	–
$K_4[^{59}Fe(^{14}CN)_6]$	3693	1.5	11.1	16	60
$KFe[^{59}Fe(^{14}CN)_6]$	4549	3.6	8.2	39	44
$Fe_4[^{59}Fe(^{14}CN)_6]_3$	4144	3.3	6.6	35	35

[1] Calculated from the exhaled dose of $^{14}CO_2$ (applicated dose of cyanide, *e.g.* 10 mg $KFe[Fe(CN)_6] \equiv 4.55$ mg cyanide = 100%).

[2] Calculated from the amount of "free" ^{14}C in urine (^{14}C-activity − ^{59}Fe-activity from hexacyanoferrate(II) compounds containing $[^{59}Fe(^{14}CN)]$) and the applicated dose of cyanide.

[3] Calculated from exhaled dose equivalent to cyanide, assuming that 33% of absorbed cyanide is expired as CO_2 within 24 h after oral application.

[4] Calculated from the excreted dose of "free" cyanide, assuming that 66% of absorbed cyanide is found in the urine within 7 days after oral application.

a substantial fraction of the cyanide was not bound to iron as $[Fe(CN)_6]^{4-}$, but excreted after release from the hexacyanoferrate(II) anion probably as CN^- or SCN^-.

A schematic summary of the absorption and excretion of iron and cyanide from oral hexacyanoferrates(II) is shown in Fig. 2.

Assuming that about 1/3 of the absorbed "free" cyanide is exhaled as CO_2 and HCN, and 2/3 are excreted in the urine mainly as rhodanide [21], then, after oral administration of 36 mg hexacyanoferrate(II)/kg body weight in rats, about 16–60 µg free cyanide/kg body weight is absorbed from a single dose of hexacyanoferrate(II) in maximum (Table VI).

As these roughly estimated values are about 70–270 times below the lethal dose of 4.3 mg CN^-/kg body weight in rats [22], no cyanide intoxication after single doses up to 100 mg/kg of hexacyanoferrates(II) have to be expected. Because of the fast detoxification rate of cyanide, *e.g.* 2.4 mg CN/kg/h in guinea-pigs [23], a toxic effect due to chronic cyanide poisoning after prolonged oral application of hexacyanoferrates(II) is also improbable.

In conclusion, the bioavailability of iron and cyanide from oral administered purified hexacyanoferrates(II) was found to be very low in rats. Since oral Prussian blue treatment has been done already in humans in the case of accidental radiocaesium contaminations and in thallium intoxications [24], a low bioavailability of cyanide from hexacyanoferrate(II) is very likely in humans, too. However, prior to a widespread use of these compounds in humans the transcription of the results in rats to human conditions has to be demonstrated. In addition, it is necessary to establish detailed specifications for the purity and the caesium binding capacities of the technically produced hexacyanoferrates(II). With regard to a long-time treatment with oral Prussian blue, further studies in domestic animals are needed.

Acknowledgement

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