CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-626

PHARMACOLOGY REVIEW

Review and Evaluation of Pharmacology and Toxicology Data

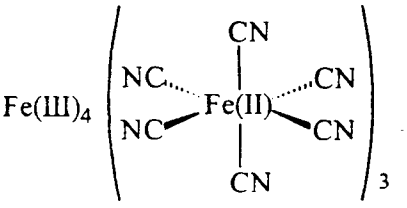
Division of Medical Imaging and Radiopharmaceutical Drug Products
HFD-160

Reviewer: Adebayo, A. Laniyonu, Ph.D.

Chemical Name: Ferric(III) hexacyanoferrate(II) $Fe_4[Fe(CN)_6]_3$

CAS Number: 14038-43-8

Structure:



Molecular Weight: 859.3

Relevant IND's: ——— 51700

Drug Class: Radioprotectants; decorporation agent

Indication: Enhancement of cesium and thallium excretion from the body.

Clinical Formulation (and components): Capsules contain 500 mg ferric hexacyanoferrate as insoluble colloids. To be administered as a 3-9 g daily in divided doses depending on ingested activity.

Route of Administration: Oral

Executive Summary

Recommendations

Prussian blue is approvable from preclinical pharmacology and toxicology perspectives. It is indicated for treatment of internal contamination from radioactive and non-radioactive cesium or thallium.

Recommendation for post-approval non-clinical studies:

[redacted]

Recommendation on labeling:

Please refer to label as written by the FDA review team.

Summary of Nonclinical Findings

Radiocesium is of great public health interest because it is a common fission byproduct, a frequent active component of sealed sources and an important radionuclide in radiation oncology. ^{137}Cs (physical $t_{1/2}$, radioactive decay constant of 30 years) decays by β emission accompanied by its daughter-product emission of photons (E=662 keV). ^{137}Cs has a high impact on human health because it is easily absorbed by the body through different routes of intake and has a relatively long biological half-life (elimination from the body) of 100 days and a physical half-life of 30 years. It is distributed uniformly throughout the body and emits β and penetrating γ radiation. For these reasons, internal deposition of ^{137}Cs will irradiate the whole body causing radiation injuries. Therefore, protection against radiocesium incorporation cannot be overemphasized. The treatment objective is rapid removal of radioactivity from the body and reduction of effective half-life (which is a function of both the physical and biological half lives) leading to a reduction in radiation absorbed dose. In view of the fact that radiocesium is secreted into the bile and reabsorbed from the intestine, an agent that reduces its intestinal absorption is expected to be an effective antidote.

Prussian Blue (PB); ferric hexacyanoferrate (II) $(Fe_4[Fe(CN)_6]_3$ has a high affinity for radioactive and non-radioactive cesium and thallium. When administered orally, it binds to either cesium or thallium in the gastrointestinal tract by ion exchange mechanisms forming insoluble complexes, thereby interrupting the entero-hepatic cycling of cesium or thallium. This results in increased elimination of cesium and thallium in feces. Overall effect is a reduction in radiation exposure to internal organs. In various animal species including rats, pigs and dogs, orally administered PB lowered the body burden level of ^{137}Cs by enhancing the fecal excretion of ^{137}Cs given orally or parenterally. PB was still effective even when there was a lag time between when treatment was started and time after Cs or thallium ingestion. However, to achieve maximum reduction in whole body radiation absorbed dose, it is essential to begin treatment as soon as possible. To a large extent, the net effectiveness of PB is proportional to the duration of treatment and dose administered. These results fit the conceptual framework that PB binds Cs^+ in the gut interrupting its entero-hepatic cycle. Presumably, PB has the potential to bind any

radioisotope that undergoes entero-hepatic cycling. PB was also effective in significantly reducing the secretion of radiocesium into ovine milk.

PB is not pharmacologically active and is essentially non-absorbable from the gastrointestinal tract. When given as a single dose orally, about 99% of the administered dose was excreted in feces with minimal amounts of iron and of cyanide ions (complexed or free) absorbed. Whether the amount of iron and or cyanide absorbed becomes significantly greater following repeated administration of PB has not been examined in detail. However, this seems unlikely considering the fact that for cyanide ions to be released, PB has to dissolve and be in a high mineral acid environment. Both of these conditions are unlikely to be encountered. Moreover, there has not been report of cyanide-related toxicity in any of the clinical or animal studies examined.

The use of PB in animals has been associated with little or no toxicity in studies reported in the literature. However, summary of a previously unpublished report is suggestive of the fact that histological changes occur in the kidney following repeated administration of PB.

In conclusion, the issue of whether pharmacology/toxicology studies of PB typically conducted and considered critical to support the safety of an NDA have been reported in the literature was examined. Overall data from the literature provide substantial support for the effectiveness of PB in the treatment of internal contamination with radioactive and non-radioactive cesium and thallium.

/S/

Reviewer's Signature: _____

Adebayo Laniyonu, Ph.D.

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Team leader Concurrence: _____

Introduction/Drug History:

There are published articles concerning the clinical effectiveness of insoluble Prussian Blue (PB) in enhancing the excretion of cesium and thallium from the body. However, FDA has not granted formal approval for any indication. During the Goiania radiological accident in 1987, the agency cleared PB for compassionate use in patients that were contaminated by the radiation exposure. The efficacy of PB has been demonstrated in several cases of radionuclide ingestion by the general public, nuclear power plant workers or by nuclear or medical researchers. The treatment objective is rapid removal of radioactivity from the body and reduction of effective half-life. The purpose of this survey is to review the available scientific literature in an attempt to examine the scientific basis of use and, to determine whether Pharmacology/Toxicology studies typically conducted and considered critical to support the safety of an NDA application have been reported in the literature. The studies upon which the scientific evidences are based were not conducted with an NDA format in mind. Nevertheless, most of the articles were published in peer reviewed journals and most of the findings were reported from multiple laboratories. This review aim to (1) identify the concepts that are scientifically valid and that appear to have general support within the scientific community and (2) Identify areas where more information is required.

Previous clinical experience:

The search did not reveal any article that solely addresses the safety or included adequate safety monitoring. No serious adverse reaction to PB has been documented at clinically relevant doses (see medical officer review).

Pharmacology:

An understanding of the conceptual basis for the use, and limitation of PB as a decorporation agent to enhance the excretion of radioisotopes of cesium or in the treatment of thallium poisoning requires a brief overview of 1), How cesium and thallium are handled in the body. 2), Integration of how PB fits into the contextual evaluation of alterations in cesium excretion pattern.

Cesium:

^{137}Cs and ^{134}Cs are the two medically significant radioisotopes of cesium for potential risk of internal contamination. ^{137}Cs is a common fission byproduct, a frequent active component of sealed sources, and an important radionuclide in radiation oncology. ^{137}Cs ($t_{1/2}$ 30 years) decays by β emission, and its daughter-product emission of photons (E=662 keV) accompanies its spectrum of radioactive decay. ^{134}Cs ($t_{1/2}$ =2.1 years) decays by both β and γ emissions, with multiple energy levels for each mode of decay.

Cesium enters the systemic circulation through either the respiratory or gastrointestinal system. Absorption from the gut is rapid for subjects in the post absorptive state. Whole body activity increases during the first four hours following ^{137}Cs ingestion and remains fairly stable until excretory and secretory losses begin. In addition, cesium follows an entero-enteric cycle. It is secreted into the intestine in the bile and reabsorbed from the

gut, and then secreted again into the gastrointestinal tract. Time course of whole body retention of inhaled ^{137}Cs is similar to that obtained following parenteral administration in rats. ^{137}Cs is distributed throughout the body and follows the movement of potassium in the body. It competes with potassium for transport across cell membrane. ^{137}Cs retention in humans can be described by the linear sum of two exponential functions. The first component usually represents up to 20% of the initial body burden, and is characterized by a biological half-life of roughly one-day. The remaining component, overwhelmingly important as regards radiation dose, is characterized by a biological half-life of from 40-140 days in adult human beings.

^{137}Cs is excreted through glomerular filtration in kidneys and in feces due to excretion via the biliary system. Urinary-to-fecal excretion ratio is species dependent. The ratio varies in humans from about 2-11.

^{137}Cs has a high impact on human health because it is easily absorbed by the body through different routes of intake and has a relatively long biological half-life of 100 days and a physical half-life of 30 years. It emits β and penetrating γ radiation and is distributed uniformly throughout the body. For these reasons, internal deposition of ^{137}Cs will irradiate the whole body causing deterministic effects and or stochastic effects.

Thallium:
Thallium is a heavy metal originally used as a depilatory agent. Its use as a potent rodenticide has been associated with accidental and intentional intoxication. Treatment of acute thallium poisoning is directed to enhancement of its elimination from the body. Thallium is rapidly absorbed from the gastrointestinal tract, and can be detected in urine and feces within one hour. Following absorption, the toxicokinetics of thallium can best be described by a 3 compartment model:

1. A central compartment consisting of the blood as well as perfused peripheral organs.
2. Brain compartment.
3. A compartment consisting of the intestine as well as the intestine content. Absorption and secretion take place since thallium undergoes extensive entero-enteral cycling.

Thallium is extensively distributed throughout the body, its elimination is slow giving rise to a long half-life in the body. The main excretory pathways are fecal and urinary.

Pharmacological Studies:

Nigrovic V., (1993): Enhancement of the excretion of radiocesium in rats by ferric cyanoferrate (II) Int. J. Rad. Biol., 7, 307-309

The study evaluated (1) the efficacy of ferric cyanoferrate (II) on the enteral absorption of ^{137}Cs. (2). The effectiveness of oral ferric cyanoferrate (II) on the elimination of parenterally administered ^{137}Cs.

Carrier free ^{137}Cs (amount not specified) and ferric cyanoferrate (II) ($Fe_4[Fe(CN)_6]_3$ were administered separately by a gastric tube to fasted (20 hours) rats (184-238 g, sex not specified). Retention of ^{137}Cs was assayed by whole-body counting four days after the administration of $^{137}CsCl$. Dosages and time schedules of administration of ferric cyanoferrate (II) are indicated in table 1. A second experiment evaluated the effectiveness of oral ferric cyanoferrate (II) on the elimination of intraperitoneally

administered ^{137}Cs. Ferric cyanoferrate (II) 50 mg per rat was orally administered 6 times between days1 and 4.

Results & Conclusion:

Ferric cyanoferrate (II)		Body weight (grams)	Percentage of ^{137}Cs-dose (95 per cent fiducial limits)	Percentage of control
Dosage (mg/animal)	Times of administration (minutes after ^{137}Cs)			
0	—	219	58·1 (63·3–53·4)	100
1	2	215	9·42 (13·2–6·72)	16
10	,,	203	1·17 (1·64–0·84)	2
50	,,	186	0·57 (0·80–0·41)	1
100	,,	188	0·52 (0·73–0·37)	0·9
0	—	185	52·5 (54·1–51·0)	100
100	30	184	29·2 (36·8–23·0)	56
100	60	238	31·8 (40·2–25·2)	61

Table 1: ^{137}Cs-retention by rats (96 hours after its oral administration) as influenced by oral administration of ferric cyanoferrate (II). Five animals per group.

Ferric cyanoferrate (II) 1-100mg/animal produced a significant reduction in the enteral

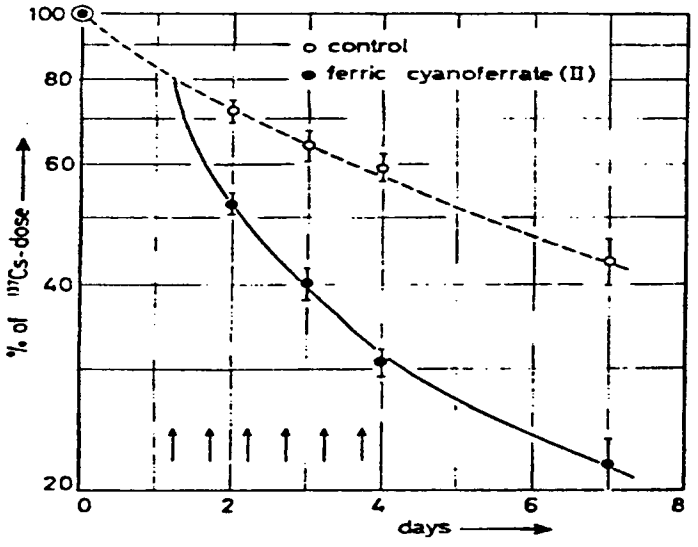


Fig 1: retention of intraperitoneally injected ^{137}Cs by rat as influenced by six times repeated oral administration of 50 mg ferric cyanoferrate (II) as indicated by arrows. ^{137}Cs injected on day 0. Eight animals per group

absorption of ^{137}Cs even when given as late as 60 minutes after ^{137}Cs administration. Figure 1 showed that orally administered ferric cyanoferrate was also effective in accelerating the excretion of intraperitoneally administered ^{137}Cs.

Reviewer's comments:

I agree with the study conclusions that orally administered ferric cyanoferrate was effective in enhancing the excretion of ^{137}Cs that was administered either orally or parenterally. The effectiveness of ferric cyanoferrate was still apparent even when administered sixty minutes after oral administration of ^{137}Cs. This is significant since there are likely scenario of delayed access to treatment following ^{137}Cs accidents. It would have been useful to know whether ferric cyanoferrate would still be effective at longer time period (greater than 60 minutes) after animal exposure to ^{137}Cs. That PB was effective in reducing total body burden of ^{137}Cs following parenteral administration is indicative of the fact that ^{137}Cs undergoes entero-hepatic circulation followed by trapping by PB in the gut.

Nigrovic, v. (1965): Retention of radiocaesium by the rat as influenced by Prussian Blue and other compounds. Phys. Med. Biol., 10 81-91

The main objective of the study was elucidation of the efficacy pattern of ferric ferrocyanide and other insoluble metal salts of ferrocyanic acids.

Carrier free ^{137}Cs (2 µCi; pH 2.5) was administered to albino rats of both sexes fasted for 20 hours prior to oral or intraperitoneal injection of ^{137}Cs. The retention of ^{137}Cs was followed by whole-body counting and expressed either as percentages of administered ^{137}Cs or as percentages of control, i.e., the ^{137}Cs content of untreated animals. Number of rats per experimental group varied from 6-10. Prussian Blue was administered at different time intervals following ^{137}Cs administration.

Results & Conclusions:

There was no significant difference between the efficacy of PB prepared from sodium and from potassium ferrocyanide. A reduced effectiveness was observed for the other metal salts of ferrocyanic acid.

Compound	Body burden (% of control and fiducial limits P=0.05)
PB (K)	1.0 (0.6-1.6)
PB (Na)	1.4 (1.1-1.7)
CoCF	4.9 (1.1-1.7)
CuCF	8.2 (3.9-17.2)
NiCF	2.4 (1.2-5.1)
ZnCF	48.0 (23-101)

Table1: Influence of PB prepared either from potassium or sodium-ferrocyanide and other metal salts of ferrocyanic acid on the enteral absorption of orally administered ^{137}Cs. 50 mg of each compound were administered orally after ^{137}CS.

Water soluble potassium ferrocyanide was less effective compared with PB in lowering the absorption of ^{137}Cs (fig 1).

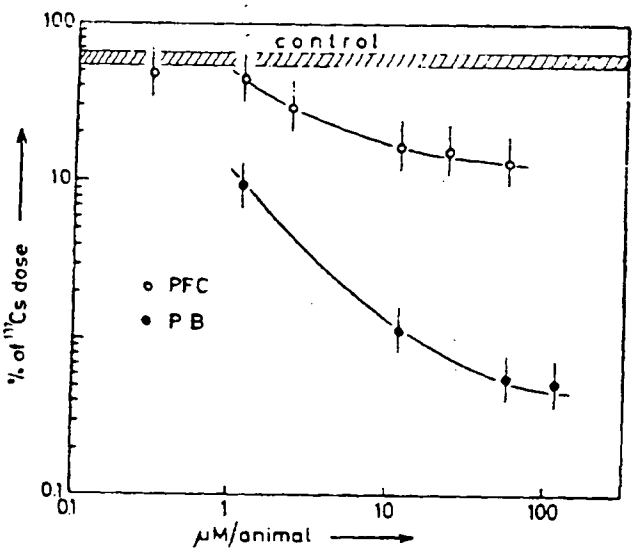


Fig 1: Influence of different doses of potassium ferrocyanide (PFC) and Prussian Blue on body burden of ^{137}Cs measured on day 4. Both compounds were given orally immediately after oral administration of ^{137}Cs. The vertical bars indicated fiducial limits

PB continuously administered in food and drinking water lowered body burden level of ^{137}Cs. Potassium ferrocyanide was less effective as the doses were increased. The author ascribed this to the significantly lowered water consumption by the animals

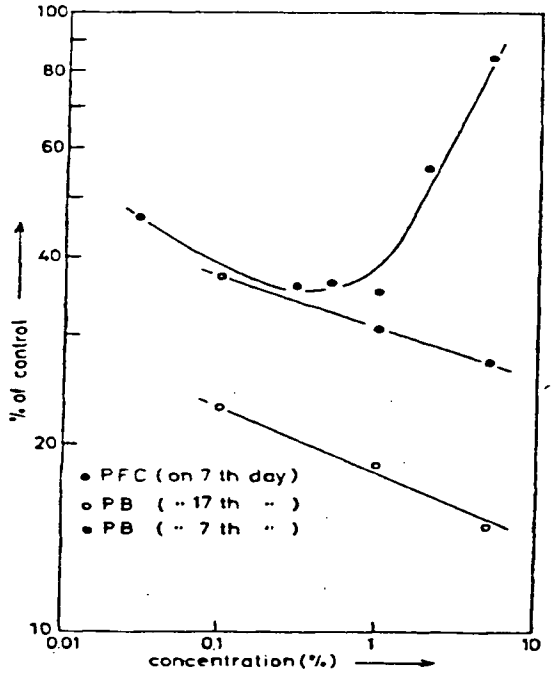


Fig 2: Influence of PB and potassium ferrocyanide (PFC) offered at varying concentrations with food and drinking water on the body burden of ^{137}Cs. Treatment was started immediately after intraperitoneal injection of ^{137}Cs. ^{137}Cs burden evaluated on day 7 (PFC) or day 7/17 (PB)

although the result of water consumption was not provided.

When treatment schedule was varied as indicated in fig 3, the slope of ^{137}Cs retention curves was identical in all PB-treated groups, and was independent of the time when treatment was started. After cessation of treatment, the curves ran parallel to control

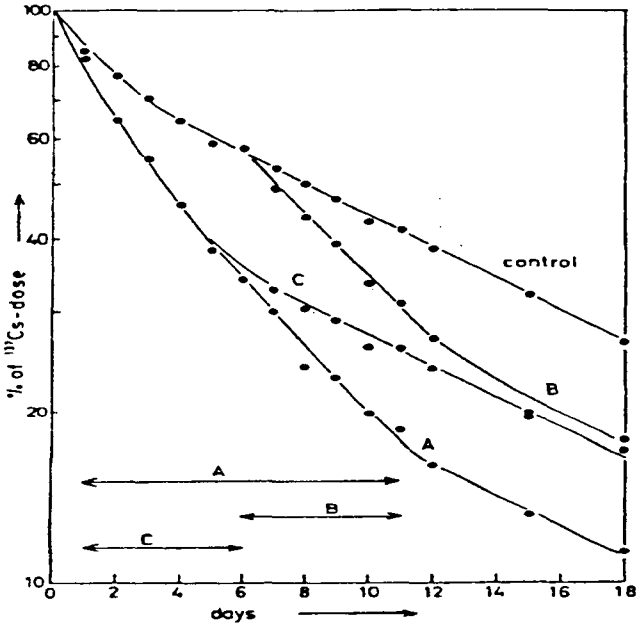


Fig 3: Influence of oral PB (50 mg) on the retention of intraperitoneal administered injected ^{137}Cs. PB was given twice daily. The duration of treatment is indicated by arrow

Reviewer's Comments:

I agree with study results and conclusion. It is interesting to note that the effectiveness of PB remains constant irrespective of the time when treatment is started, and that the net effect is proportional to the duration of treatment and total amount of PB administered. PB was still effective even when treatment was delayed up to 6 days after ^{137}Cs ingestion. The efficacy of the water soluble potassium ferrocyanide was less than that of PB.

Ioannides, K.G., Mantzios, A.S., & Pappas, C.P. (1991): Influence of Prussian Blue in reducing transfer of radiocesium into ovine milk: Health Physics 60, 261-264

The study examined the effectiveness of ferric ferrocyanide in reducing the secretion of radiocesium into ovine milk.

Ten ewes in late lactation (~35kg, average daily milk yield ~110g were fed radiocontaminated wheat (mean radiocesium concentration 1684 ± 17 Bq/kg) In addition, each animal was offered approximately 200 g of highly contaminated dried grass (9840 ± 442 Bq/kg) for 2 months. Radiocesium concentration in milk was monitored during this period. When the contamination in milk approached equilibrium,

the ewes were assigned to two groups; treated and control. Treated group ewes were offered PB in their drinking water 1 g/l for 23 days during which milk samples were collected for analysis.

Results & conclusion:

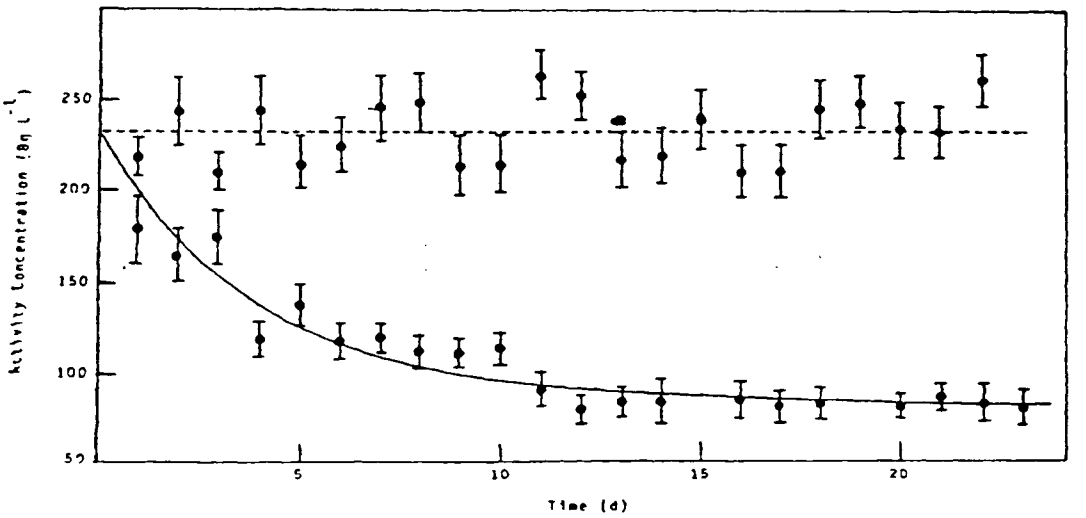


Fig 1: Measurements of radiocesium activity concentration in milk following the administration of PB. Dashed line corresponds to average contamination measured in milk of control animals.

Radiocesium was secreted into milk at a constant rate (fig 1). Introduction of PB into ewes diet decreased the concentration of ^{137}Cs activity in milk by approximately 85%. PB did not affect the elimination rate constant of cesium.

Reviewer's comments: I agree with the study results. The influence of PB in reducing the concentration of radiocesium in milk was apparent as soon as PB treatment began and reached a nadir by about day 12 of treatment. It is not clear from the study whether increasing the concentration of PB would have resulted in further reduction ^{137}Cs activity in milk. The fact that the elimination rate constant of ^{137}Cs was not affected is consistent with the fact that PB is not absorbed into the body or interacts with blood to remove extracellular or intracellular ^{137}Cs.

Dresow, B., Nielsen, P., Fischer, R., Pfau, A.A. & Heinrich, H.H. (1993): in vivo binding of radiocesium by two forms of Prussian blue and by ammonium iron hexacyanoferrate (II): Clinical Toxicology, 31, 563-569

The study examined the effect of two forms of Prussian Blue, soluble $K_3Fe[Fe(CN)_6]$ and insoluble $Fe_4[Fe(CN)_6]_3$, and of ammonium iron hexacyanoferrate (II) $(NH_4Fe[Fe(CN)_6]$ on intestinal radiocesium absorption in rats, pigs and humans (only rats and pigs studies will be discussed)

Fasted female Wistar rats (260-280 g, n=10) were administered 5 mg Prussian Blue or the other antidotes 2 minutes before $^{134}CsCl$ (11.1- 29.6 KBq). Control animals received $^{134}CsCl$ only. $^{134}CsCl$ whole-body activity measured immediately after ^{134}Cs application was taken as reference value. Rats were housed in metabolic cages and urine collected for seven days. Thereafter whole-body retention and ^{134}Cs in urine were measured.

For the pig study, pigs (82 ± 2 kg) were fed radiocesium-contaminated diet for 27 days. Prior to each feeding, 0.5, 1.5, or 2.5 g of KFeHCF, FeHCF, or NH_4FeHCF was administered in gelatin capsules. Control pigs did not receive antidote. After the 27 day feeding period, the animals were slaughtered and samples (without bone) were taken from shoulder, chop, and ham and counted for radioactivity.

Results and Conclusions:
For the rat study, the 3 compounds used showed a high in vivo adsorption capacity for cesium resulting in an almost complete blockade of radiocesium absorption from the GI as judged by urinary excretion and whole body retention 7 days after application.

TABLE 1

Prevention of Intestinal Radiocesium Absorption in Rats* by Prussian Blue

Compound†	Urinary excretion‡	WBR§	Absorption¶
Control	44.0 ± 3.50	40.7 ± 1.04	84.7
FeHCF	3.04 ± 0.85	3.32 ± 1.57	6.36
KFeHCF	1.86 ± 1.72	0.77 ± 0.49	2.63
NH_4FeHCF	1.77 ± 0.37	0.66 ± 0.53	2.43

*n = 10
†5 mg of Prussian blue or NH_4FeHCF was administered through a gastric tube 2 min before oral ^{134}Cs (carrier-free, 11.1 - 29.6 KBq), control without antidote
‡accumulated urinary excretion, % of dose 7 days after loading, mean ± SD
§whole-body retention; % of dose 7 days after loading, mean ± SD
¶% of dose 7 days after loading

Similar results were obtained in the pig study (table 2). Administration of increasing amount of the antidotes resulted in a dose-dependent reduction in activity concentration in all samples tested. Insoluble FeHCF was less effective

TABLE 2

$^{134/137}$Cs Activity Concentration in Pork (weighted mean) of Pigs Receiving Radiocesium-Contaminated Whey*

Test Series	Prussian Blue Dosage g/d	Pork (ham+shoulder+chop)† Bq/kg
Control‡	0	359 ± 42
KFeHCF§	1	69 ± 10
	3	24 ± 6
	5	11 ± 1
FeHCF§	1	263 ± 25
	3	128 ± 16
	5	29 ± 5
NH_4FeHCF§	1	75 ± 9
(Giese-salt)	3	26 ± 6
	5	20 ± 5

*2 x 200 g/d; $^{134/137}$Cs, 1890 Bq/d for 27 days
†mean ± SD; SD includes statistical measurements errors and biological deviations
‡n = 10, no antidote
§n = 2 for each antidote dose

The study concluded that all three compounds tested are potent antidotes for the efficient inhibition of radiocesium absorption.

Reviewer's comments: I agreed with study conclusion.

Heylauf, H. (1969): Ferric cyanoferrate(II), an effective antidote in thallium poisoning. Eur J Pharmacol 6 340-344

The study examined the influence of orally administered Prussian Bue on the distribution and excretion of ^{204}Tl in rat.

Male rats (~260 g) were administered carrier free ^{204}Tl(I) (0.5 ml, pH ~ 3, 2.5-5 µCi per animal, specific activity 4.7 mCi.mg^{-1}) by gastric tube or into the tail vein. PB was administered by gastric tube. For the animals that received ^{204}Tl(I) intravenously, PB-pellets were given ad libitum up to the 9th day after ^{204}Tl(I) The animals were housed in special metabolic cages for urine and feces collection. Tissues and excretory matter were counted for activity.

Results and conclusions:

PB was maximally effective when given simultaneously with ^{204}Tl(I) although a distinct effect was still observed with PB administered 60 minutes after. PB effect was dose dependent (table 1).

PB-dose (mg)	Time of administration (min)	n	% of ^{204}Tl-dose				
			Liver	Kidneys	Skeleton	Muscles	Testes
-*	-	6	2.11 ± 0.13	5.95 ± 0.31	4.25 ± 0.23	47.8 ± 0.83	1.09 ± 0.080
-	-	12	2.02 ± 0.069	4.82 ± 0.14	3.86 ± 0.097	41.3 ± 0.83	1.10 ± 0.028
0.5	<1	5	1.50 ± 0.13	3.69 ± 0.15	3.08 ± 0.20	27.5 ± 1.21	0.83 ± 0.054
5	<1	6	0.90 ± 0.084	2.31 ± 0.25	1.75 ± 0.17	17.8 ± 1.77	0.49 ± 0.097
50	<1	17	0.21 ± 0.025	0.51 ± 0.057	0.41 ± 0.043	4.36 ± 0.43	0.12 ± 0.012
50	10	5	1.01 ± 0.13	2.45 ± 0.36	1.92 ± 0.29	19.7 ± 2.40	0.55 ± 0.10
50	30	6	1.29 ± 0.15	2.88 ± 0.39	2.35 ± 0.29	24.0 ± 2.47	0.78 ± 0.097
50	60	5	1.48 ± 0.16	3.64 ± 0.16	2.53 ± 0.22	25.2 ± 1.43	0.83 ± 0.052

Table 1: ^{204}Tl-content of organs 48 hr after oral administration of ^{204}Tl as influenced by dose and time of administration of PB. Mean average ± S>E. n = number of animals. * ^{204} Tl intravenously.

Orally administered PB was effective against intravenously administered ^{204}Tl(I) (Fig 1).

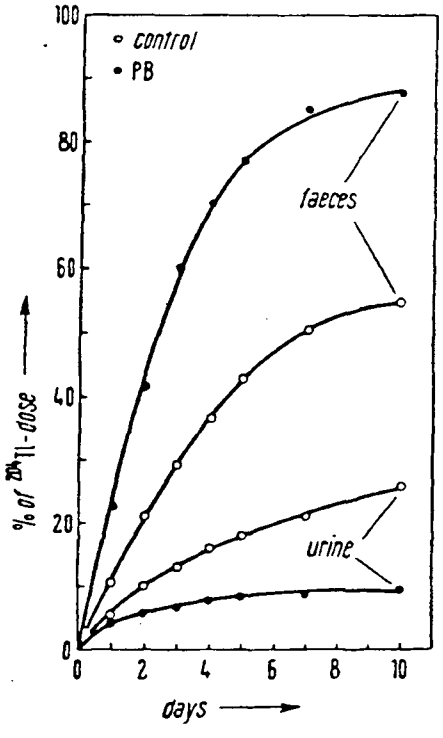


Fig1: Cumulative excretion of intravenously injected ^{204}Tl as influenced by feeding of PB-pellets. 5 animals per group.

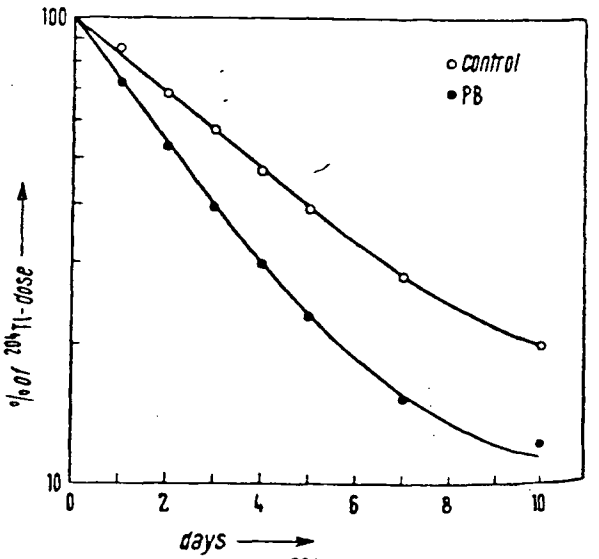


Fig 2: Diminution of the ^{204}Tl-body burden with time. The body burden was assumed to equal 100% - net excretion.

The body burden as calculated from the cumulative net excretion decreased during the first five days exponentially (fig 2), the half-life in the PB- and the control series being 2.2 and 3.8 days respectively.

The study concluded that PB markedly lowered the retention of ^{204}Tl(I) by inhibiting its absorption and reabsorption from the GI tract.

Reviewer's Comments:

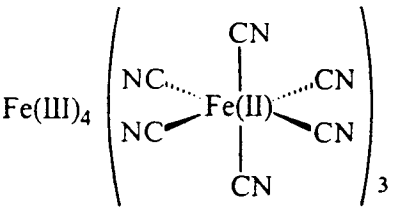
I agree with the study conclusions. The fact that orally administered PB was effective against ^{204}Tl(I) given intravenously is indicative of the fact that PB can interrupt the enteral recycling of Tl(I). PB was also effective even when therapy was delayed.

Pharmacokinetics:

Nielsen, P., Dresow, B., Fischer, R., Gabbe, e.E., Heinrich, H.C. & Pfau, A. (1988): Intestinal absorption of iron from ^{59}Fe-labeled hexacyanoferrates (II) in piglets: Drug Research, 38, 1469-1471

The study objective was to determine the intestinal absorption of ^{59}Fe and ^{14}C from hexacyanoferrates (II) in piglets.

$KFe^{III}[Fe^{II}(CN)_6$ **(I)** and $Fe^{III}_4[Fe^{II}(CN)_6]_3$ **(II)** were labeled with ^{59}Fe both in the Fe(II) position (outside the complex anion, **a**) or in the Fe(II)-position (hexacyanoferrate anion, **b**, inside the complex)



Piglets of both sexes (weight 6.9 ±1.0 kg) were randomized to different experimental groups and fasted for 4 hours prior to oral administration. 50 ml solution containing 0.3 mmol I (0.1 mmol II) of the respective ^{59}Fe (and ^{14}C) labeled hexacyanoferrate (34-39 mg Fe; 96-221 kBq ^{59}Fe; 3-5.2 MBq ^{14}C was administered by gastric intubation. ^{59}Fe retention was measured immediately after administration, and at 7 & 14 days after application by whole body counting. Two piglets from each group were kept in metabolic cages for seven days to measure ^{59}Fe in urine and feces. Expired air $^{14}CO_2$ was measured in two piglets that received $Fe_4[^{59}Fe^{II}(^{14}CN)_6]_3$.

Results and conclusion:

1:
Results of the study showed very poor absorption of ^{59}Fe labeled either in the Fe(III) position or the Fe(II) position (tables 1 & 2).

$K^{59}Fe[Fe(CN)_6 2H_2O$	$KFe[^{59}Fe(CN)_6 2H_2O$	$^{59}Fe_4[Fe(CN)_6 15H_2O$	$Fe_4[^{59}Fe(CN)_6 15H_2O$
1.47±0.31	0.20±0.03	1.34±0.39	0.15±0.05
N=8	N=11	N=8	N=11

Table 1: % ^{59}Fe-whole body retention 14 days after oral application of $K\,Fe^{(III)}[Fe^{(II)}(CN_6]$ or$Fe^{(III)}_4[Fe^{(II)}(CN)_6]_3$ labeled either in the Fe(III) or Fe(II) position

	$K^{59}Fe[Fe(CN)_6 2H_2O$		$KFe[^{59}Fe(CN)_6 2H_2O$		$^{59}Fe_4[Fe(CN)_6 15H_2O$		$Fe_4[^{59}Fe(CN)_6 15H_2O$	
	PAU 03	PAM 04	PAM 07	PAM 05	PAM 09	PAU 1	PAU 12	PAU 13
Feces	103	81	98	99	93	102	99	110
Urine	0.3	1.0	0.2	0.6	1.0	0.6	0.1	0.1
Whole body retention	1.3	0.3	0.1	0.4	0.9	0.03	0.1	0.1
Recovery	105	82	99	100	95	103	99	110

Table 2: Cumulative ^{59}Fe (% of administered dose) in feces and urine of piglets receiving oral doss of different ^{59}Fe-labeled hexacyanoferrates (II). Piglets were kept in metabolic cages for 7 days.

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The $^{14}CO_2$ activity in the expired air of pigs that received $Fe_4[^{59}Fe(^{14}CN)_6]_3$ was below the detection limit of the measuring system.

The study concluded that low amount of iron and of cyanide ions (complexed or free) are absorbed from insoluble and soluble Prussian Blue and that these agents can be

used without reservation in the prevention of radiocesium and/or decoporation of radiocesium in domestic animals and humans.

Reviewer's comments:

I agree with the study conclusions that low amounts of iron and of complexed and /or free cyanide ions are absorbed from orally administered single dose PB. However PB will be used more than once in the same patient/subject, and it would have been helpful if pattern of absorption was studied after repeated administration of PB.

Toxicology:
Short-term feeding study of sodium ferrocyanide in rats.
Summary of an unpublished report (dated 11/30/1959) by the Food and Drug Research Laboratories inc., USA, commissioned by the International Salt Company Inc, USA
Fd Cosmet. Tox 7 409-410 (1969)

Study no: Not applicable.
Volume #, and page #: Not applicable.
Conducting laboratory and location: Not stated.
Date of study initiation: Not provided.
GLP compliance: No (study predate GLP regulation)
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: Not stated
Formulation: Sodium ferrocyanide in —— chow.diet.

Methods: Groups of ten males and ten female rats (weight not specified) were fed for 90 days on —— laboratory chow containing 0, 0.05, 0.5 or 5 % sodium ferrocyanide equivalent to 25, 250, 2500 mg/kg/day.

Observations and Times:

Clinical signs: Not stated
Body weights: weekly
Food consumption: weekly
Hematology/clinical chemistry: day 90
Organ weight: At necropsy
Histopathology: End of study

Results:
Clinical signs: Not stated

Body weight: weight gain was said to be normal with the exception of the males in the 5% group in which weight gain was reduced significantly (significant level not stated)

Food intake: Food intake was said to be normal but that efficiency of food utilization (weight gain/food consumed) was impaired at the 5% level in both sexes

Hematology and blood chemistry: hemoglobin and hematocrit values were depressed in male and female rats in the 5% group. Other groups were normal. All groups displayed normal total and differential leucocyte counts and normal blood glucose and non-protein nitrogen.

Mortality: A male rat in the 5% sodium group died after 10 weeks.

Organ weight: enlargement of the kidneys occurred at the 5% level in both sexes and at0.5% level in females. Male adrenals and female pituitaries were also enlarged at the 5% level

Gross and histological findings:
Gross: significant changes were seen only in the kidneys; paleness, hydronephrosis and crystalline deposits were evident especially at the 5% level. Histological changes seen at 0.5 and 5 % levels include epithelial damage of focal segments of the midcortical convoluted tubules characterized by the presence of irregular nuclei with abnormal chromatin patterns. In addition, the 5% group exhibited calcified deposits in the renal pelvic epithelium, and the mucosa showed areas of squamous metaplasia. According to the report, the changes observed may have been secondary to the accumulation of crystalline deposits tentatively identified as urates and oxalates. Tests for ferrocyanide was said to be negative. Crystalline depositions were said to be associated with hydronephrosis.

Conclusion: NOEL was defined as 0.05% equivalent to 25 mg/kg/day.

Reviewer's comments:
The results of this study suggest that PB might be absorbed following chronic administration as manifested by systemic toxicity. The kidneys seem to be the main target of toxicity, perhaps due to deposit of PB crystals in this organ. While the study of Nielsen and colleagues suggested that PB absorption was negligible, it bears emphasizing that the study examined a single dose of labeled PB. In view of the potential for kidney damage with chronic administration of PB, and lack of any other detailed comprehensive toxicological study, the reviewer recommends that the issue of the toxicity of PB administered chronically be examined in a large animal species.

Carcinogenicity:

Study addressing the carcinogenicity of PB was not identified, Such studies would not be expected to be conducted.

Immunotoxicology:

Study addressing the immunotoxicity of PB was not identified, Such studies would not be expected to be conducted.

Reproductive Toxicology:
PB is not absorbed from the gastrointestinal tract; therefore reproductive toxicologic effects or appearance in milk is not expected.

Genotoxicity

Study addressing the genotoxicity of PB was not identified, Such studies would not be expected to be conducted.

Conclusions and Recommendations:

Prussian Blue is approvable from preclinical pharmacology and toxicology perspective. Please refer to the overall executive summary and individual study evaluation.

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/s/

Patricia Stewart
9/4/03 05:18:43 PM
CSO