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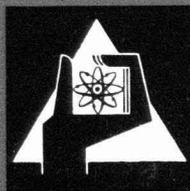
Institut für Strahlenbiologie

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Prussian Blue and Other Compounds

V. Nigrović

Gesellschaft für Kernforschung m. b. H.
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**GESELLSCHAFT FÜR KERNFORSCHUNG M. B. H.
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Retention of Radiocaesium by the Rat as Influenced by Prussian Blue and Other Compounds

By V. NIGROVIĆ, M.D.

Institut für Strahlenbiologie, Kernforschungszentrum, Karlsruhe, Germany

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§ 1. INTRODUCTION

^{137}Cs must be considered as a potentially hazardous radionuclide and a considerable amount of work has been done aiming at the enhancement of ^{137}Cs -excretion. Slightly higher urinary excretion rates were observed by Kurlandskaya (1957) and Ogawa, Machida, Suzuki and Shibata (1958) following the administration of stable Cs-salts; however, Moskalev (1961 b) failed to confirm this finding. The excretion of ^{137}Cs in rats was enhanced by increasing the potassium content of the diet (Richmond and Furchner 1961 b, Wasserman, Comar and Tapper 1963); a similar effect, however, has not been observed in humans (McNeill, Green and Rapoport 1962). The failure of the chelating EDTA to raise the urinary excretion (Fateyeva, Klimov, Ponizovskaya, Gorbarenko, Sokolov and Smirnova 1960) is not surprising, due to the poor co-ordinating properties of the alkali metal ions. Continuous administration of the diuretic Diamox reduced the body burden of ^{137}Cs in small rodents to approximately 70% of the control (Richmond and Furchner 1961 a); however, in humans diuretics proved to be ineffective (Rosoff, Cohn and Spencer 1963). A detailed enumeration of all other pharmaceuticals, hormones, etc., which have been tested without positive results, can be omitted.

A promising approach is offered by the fact that the faecal excretion of ^{137}Cs is rather low (Moskalev 1961 a) despite its fairly high secretion into the intestinal tract (Moore and Comar 1962). Obviously, a reabsorption of ^{137}Cs has to be assumed and it could be anticipated that an interruption of this 'enteral cycle' would raise the faecal excretion of ^{137}Cs . In keeping with this assumption, Mraz and Patrick (1957 b) have demonstrated a distinct effect in animals maintained on a diet containing bentonite or vermiculite: with orally and parenterally administered ^{137}Cs , the body-burden was reduced to 70 and 90% of the control value, respectively. The net effect, though interesting from the theoretical viewpoint, is disappointingly small, and it is questionable whether the compounds referred to above are the optimal ones. Summing up, it can be stated that, so far, no antidotes are available for the treatment of internal contamination with ^{137}Cs .

The main accent of the present investigation lies on the search for agents able to bind effectively ^{137}Cs in the intestines and, in particular, on the

elucidation of the efficacy pattern of ferric ferrocyanide, a compound which in preliminary screening tests yielded rather promising results (Nigrović 1963).

§ 2. MATERIAL AND METHODS

Carrier-free $^{137}\text{CsCl}$ ($2\ \mu\text{c}$; pH 2.5) was administered to albino rats of both sexes either by gastric intubation or by intraperitoneal injection. The animals, 12 to 20 weeks old, were fasted for 20 hours prior to the oral administration. If not stated otherwise, the diet consisted of standard pellets (1% potassium) and water *ad libitum*. The retention of ^{137}Cs was followed by whole-body counting; a plastic scintillator (7 in. diam., 5 in. deep) was used. The activity, which was measured immediately after the administration of ^{137}Cs , was taken as 100%. Constant counting efficiency was assured by assaying standard ^{137}Cs -samples. Screening experiments demonstrated that the position of the rat in the plastic tube (6 cm diam., 17.5 cm long, wall-thickness 0.5 cm) used did not influence the counting rate. The tube-to-scintillator distance was 19 cm.

All chemicals used were of analytical grade. The different insoluble metal salts of ferrocyanic acid were prepared by addition of the corresponding metal salt to potassium ferrocyanide solution in the appropriate molar amounts. The precipitates were separated by centrifugation, washed with distilled water, dried and pulverized.

The ^{137}Cs -content of the body was expressed either as percentages of the administered ^{137}Cs -amount, or as percentages of the control, i.e., the ^{137}Cs -content of untreated animals; each single experimental series contained its own control group. The number of rats per experimental group varied from 6 to 10.

§ 3. RESULTS

The first experiments were designed to find the compounds with the greatest ability to bind ^{137}Cs in the intestinal tract, either by precipitation, absorption, or by ion-exchange. Carrier-free ^{137}Cs and, immediately thereafter, the compound to be tested were administered by gastric tube. Sodium perchlorate, sodium-potassium tartrate, sodium cobaltinitrite, zirconium phosphate, magnesium trisilicate, aluminium oxide, Graham's salt, Dowex A, and polyacrylic acid, had no significant effect on the absorption of ^{137}Cs , and a detailed presentation of the results is omitted. It may be mentioned only that the ability of Dowex A to bind ^{137}Cs *in vitro* proved to be high, so that one may assume that the binding is not stable enough *in vivo*, when the presence of numerous competing ions in the intestinal tract has to be taken into account.

As can be seen from table 1, sodium tetraphenylborate definitely reduces the absorption of ^{137}Cs . In order to be effective this compound must be administered at the same time as the ^{137}Cs . The efficacy is reduced when the administration was postponed even for 30 seconds

(experiment B, table 1), becoming virtually negligible at greater intervals (data not presented).

Table 1. Influence of orally administered sodium tetraphenylborate (TPB) on the enteral absorption of ^{137}Cs . TPB was administered either simultaneously with ^{137}Cs (experiment A) or approximately 30 sec after the oral application of ^{137}Cs (experiment B).

mg TPB per rat	Body-burden (% of dose and fiducial limits ($P=0.05$)) on the 4th day	
	exp. A	exp. B
0	60 (49-73)	60 (52-70)
0.10	54 (44-66)	69 (59-80)
0.56	27 (22-33)	62 (53-72)
1.0	20 (16-24)	50 (43-58)
3.2	39 (32-47)	42 (36-49)
8.0	38 (31-47)	38 (33-45)
16.0	44 (35-53)	—

Fig. 1 summarizes the results of the experiments where oral or intraperitoneal administration of ^{137}Cs was followed after different time intervals by oral administration of ferric ferrocyanide (Prussian Blue, PB). With orally administered ^{137}Cs , there is a sharp diminution of effectiveness during the first two hours, whereas with intraperitoneally injected ^{137}Cs the time-dependence is less pronounced. Obviously, the efficacy of PB administered after three hours does not depend upon the mode of administration of ^{137}Cs ; in both cases, the body-burden is reduced to between 80 and 90% of the control.

Fig. 2 compares the effectiveness of PB and of the water-soluble potassium ferrocyanide at different dose-levels. Potassium ferrocyanide, though effective in lowering the absorption of ^{137}Cs , is definitely inferior to PB.

On the other hand, potassium ferrocyanide (10 mg/animal) injected intravenously and simultaneously with ^{137}Cs , leads to a diminished elimination of ^{137}Cs ; the body-burden on the 7th day being $70.5 \pm 1.5\%$ as compared with $49.9 \pm 1.5\%$ in the control group.

As can be seen from table 2, there is no significant difference between the efficacy of PB prepared from sodium and from potassium ferrocyanide. Separate administration of FeCl_3 and potassium ferrocyanide (in a molar ratio of 4 : 3), which should be followed by the precipitation of PB in the stomach, is definitely less effective than PB. A reduced effectiveness was also observed for the other metal salts of ferrocyanic acid enumerated in table 2, as well as for isotopically diluted ^{137}Cs (table 3).

A further experimental series tested the effect of *continuously* administered PB on the retention of *intraperitoneally* injected ^{137}Cs . As can be seen from fig. 3, an essentially lower body-burden results from PB mixed with the food in various concentrations. Potassium ferrocyanide (given

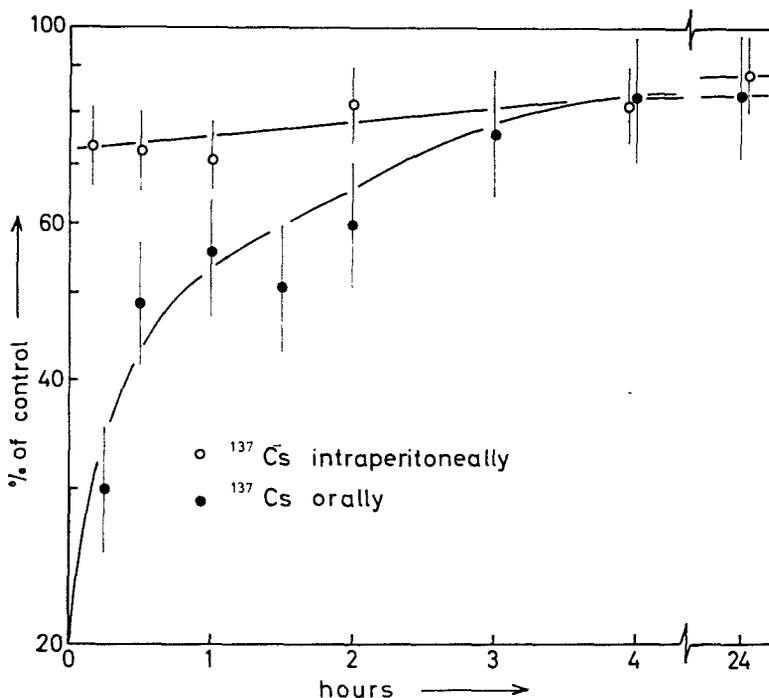


Fig. 1. Influence of 50 mg Prussian Blue (PB) administered orally after different time intervals on the body-burden of orally and intraperitoneally administered ^{137}Cs (on the 4th day). The vertical bars indicate fiducial limits ($P=0.05$).

Table 2. Influence of Prussian Blue (PB) prepared either from potassium- or sodium-ferrocyanide, and other metal salts of ferrocyanic acid (CF) on the enteral absorption of orally administered ^{137}Cs . 50 mg of each compound were administered orally immediately after ^{137}Cs .

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 (2.3-10.3)
CuCF	8.2 (3.9-17.2)
NiCF	2.4 (1.2- 5.1)
ZnCF	48.0 (23-101)

in drinking water) is, in accordance with the results presented in fig. 1, less effective. The poor effectiveness of the two highest dose-levels may be ascribed to the significantly reduced consumption of water by the rats. Also, a marked loss of body weight was noted in these groups.

The relatively long duration of the PB-experiment (i.e., 17 days) makes it possible to determine the influence of PB on the retention function of

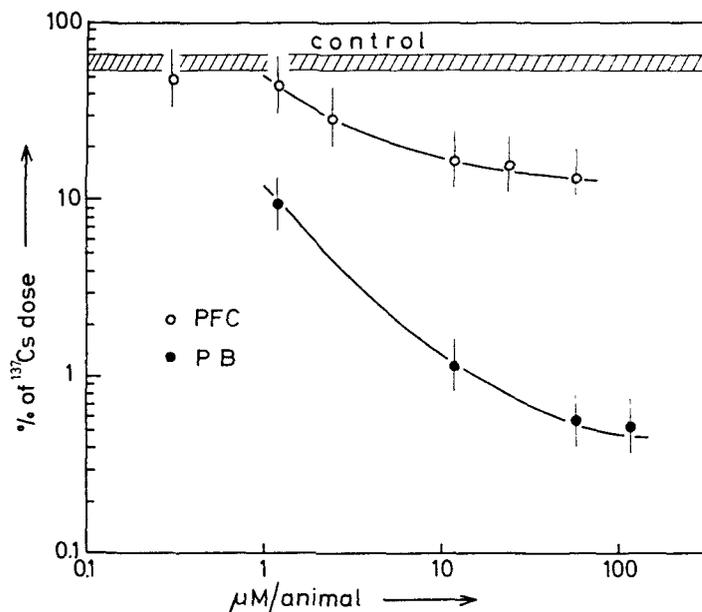


Fig. 2. Influence of different doses of potassium ferrocyanide (PFC) and Prussian Blue (PB) on the body-burden of ^{137}Cs (on the 4th day). Both compounds were given orally immediately after oral administration of ^{137}Cs . The vertical bars indicate fiducial limits ($P=0.05$).

^{137}Cs . In all the experimental groups the dependence of the body-burden (R) upon time (t , in days) can be fitted satisfactorily by the multiexponential expression

$$R = a_1 \exp(-\lambda_1 t) + a_2 \exp(-\lambda_2 t).$$

In untreated animals, a_1 equals 25% of the administered ^{137}Cs -amount and is excreted with a half-time of approximately 1 day, whereas the residual 75% (a_2) are eliminated with a half-time of 8.5 days. The administration of PB exerts, as can be seen from fig. 4, a twofold influence: it decreases a_2 and increases λ_2 . The increase of PB-dosage, however, gives rise to a slight increase of efficacy only.

Since no attempts were made in the preceding experiments to determine the consumption of food and, thus, the actual intake of PB, in another experiment PB was given by gastric tube twice daily (9 a.m. and 5 p.m.).

The treatment schedule was varied as indicated in fig. 5. The slope of the retention curves in all PB-treated groups is identical and independent of the time when treatment was started; after cessation of treatment the curves ran parallel with the control. The retention in the control group

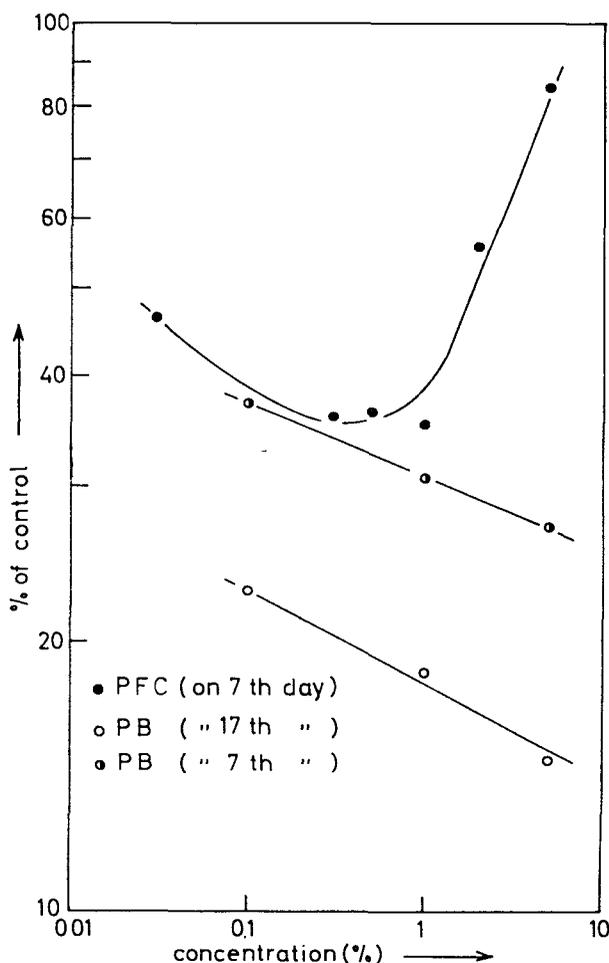


Fig. 3. Influence of PB and potassium ferrocyanide (PFC) offered at varying concentrations with food and drinking water, respectively, on the body-burden of ^{137}Cs . Treatment was started immediately after the intraperitoneal injection of ^{137}Cs .

can be described by $R = 17 \exp(-0.770t) + 83 \exp(-0.066t)$. In the group, where PB was administered beginning 1 day after ^{137}Cs -administration, the corresponding equation is $R = 18 \exp(-0.770t) + 82 \exp(-0.141t)$. This is in contrast to the preceding experiment (fig. 4), which demonstrated a significant decrease of a_2 under the influence of PB.

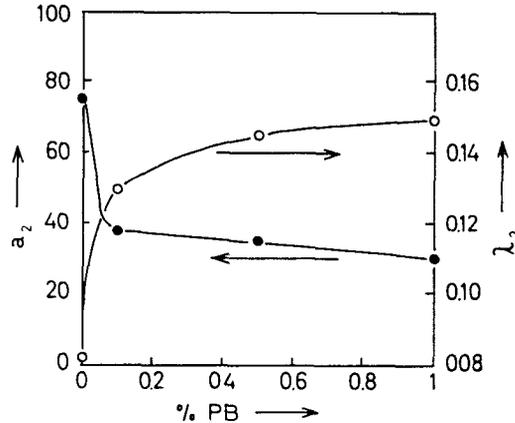


Fig. 4. Effect of different doses of PB on the multiexponential retention function of ^{137}Cs . For further explanations see text.

An essential prerequisite for obtaining a maximal effect under chronic experimental conditions is the proper spacing of multiple doses of PB. As can be seen from fig. 6 the same total dose is significantly more effective when it is divided in two equal doses separated by 2 to 4 hours.

Finally, it may be stressed that in all experiments no toxic side effects of PB were noted. Even continued treatment with high dosages (50 mg twice daily for 11 days) was well tolerated, and no loss of body-weight was observed.

§ 4. DISCUSSION

It lies beyond the scope of this study to discuss in detail the mechanism by which PB binds Cs^+ , and pertinent publications dealing with this question are referred to (Kyrsh and Zvyagintsev 1958, Pushkarev Skrylev and Bagretsov 1960, Roginskiy, Malimina, Yanovski, Altshuler and Morokhovets 1960). Since the crystal lattice of PB contains considerable amounts of potassium, ion-exchange is most likely involved*. This is compatible with the fact that an increase of the Cs-concentration, i.e., isotopic dilution of ^{137}Cs , is followed by a decrease of PB-efficacy (table 3). Although other metal salts of ferrocyanic acid bind Cs^+ , probably, in the same way as PB, the reason for their lower efficacy is not yet clear: their solubilities are distinctly higher than that of PB (Tananayev, Glushkova and Seifer 1956), but these differences are not pronounced enough to play a significant role.

The experimental results are consistent with the hypothesis outlined in the introduction, namely that PB binds Cs^+ in the gut and, thus, interrupts its 'enteral cycle'. Apparently, the presence of other cations in the gastrointestinal tract, e.g., Na^+ , K^+ or Ca^{2+} , as well as changes of the pH, do not interfere with this effect.

*The highly selective ion exchange of Cs on ferrocyanides has been substantiated by Kouřim, Rais and Million (J. Inorg. Nucl. Chim. 1964, 26, 1111).

The most likely mechanism by which the water-soluble potassium ferrocyanide exerts its influence is the *in situ* formation of PB (solubility product 10^{-40} , Tananayev *et al.* 1956) and of other insoluble ferrocyanides. Since the efficacy of potassium ferrocyanide does not

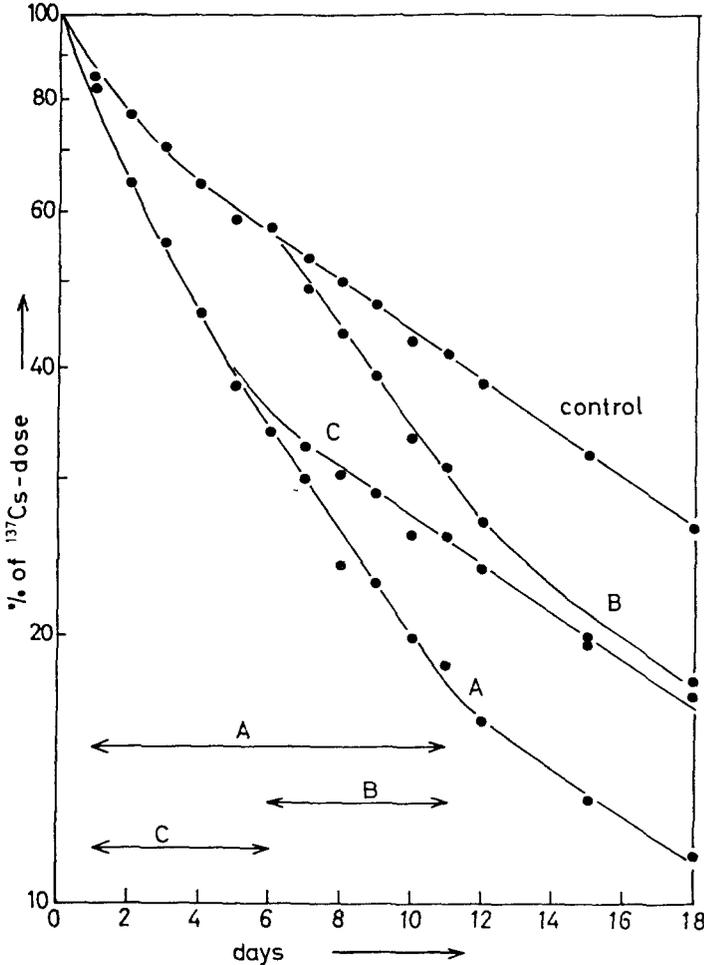


Fig. 5. Influence of oral PB (50 mg) on the retention of intraperitoneally injected ^{137}Cs . PB was given twice daily. The duration of treatment is indicated by the arrows.

reach that of PB even when FeCl_3 is administered additionally, the *in situ* precipitated material may well have a different particle size and contain absorbed material which is not present in the *in vitro* prepared compound.

In untreated animals, approximately 20% of ^{137}Cs is excreted with a half-time of 1 day and the residual 80% with a half-time of 8 to 11 days; this is in accordance with the observations of Richmond and Furchner

(1961 *a* and *b*). Provided that PB is administered early, i.e., during the first 24 hours, it exerts a definite effect on both components. Obviously, the high rate of ^{137}Cs secretion into the intestines, observed by Moore and Comar (1962) during the first hour, is of short duration only. When treatment with PB is started after a delay, only the slow compartment is influenced.

Table 3. Influence of 50 mg Prussian Blue administered orally on the enteral absorption of isotopically diluted ^{137}Cs . PB was given immediately after the oral application of ^{137}Cs .

Carrier (mg Cs)	Body-burden (% of control and fiducial limits ($P=0.05$) on the 4th day
0	1.2 (0.7- 2.1)
1	1.9 (1.1- 3.3)
5	4.9 (2.8- 8.5)
10	16.7 (9.3-28.8)
20	40.6 (23.1-71.5)
30	35.2 (20.0-61.7)

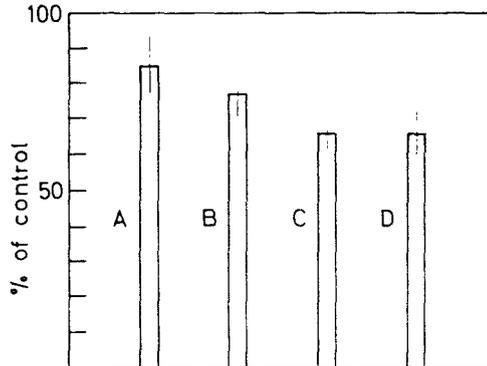


Fig. 6. Influence of orally administered PB on the body burden of intraperitoneally injected ^{137}Cs on the 4th day. 100 mg immediately after ^{137}Cs (A), 50 mg immediately and 50 mg after 2 (B), 4 (C) and 8 hours (D). The vertical bars indicate fiducial limits ($P=0.05$).

An essential feature is 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. This is valid, at least, for the first weeks following incorporation of ^{137}Cs .

Maximal efficacy can be achieved only if an *optimal* and *constant* concentration of PB is maintained in the whole intestinal tract. This is

exemplified by the results shown in fig. 6. For technical reasons (in the experiment where PB was given by gastric tube), and because of the irregular consumption of food by the rats, there can be no doubt that the largest obtainable effect was *not* achieved in our experiments. In spite of this, the actually observed effect, i.e., reduction of the body-burden by 80 to 85%, is fairly high and compares well with the results obtained with other radionuclides by the use of specific chelating agents.

In view of the high efficacy as well as the complete lack of any toxic side effects, PB must be considered as a valuable antidote in cases of accidental internal contamination with ^{137}Cs . As to the usage of PB in humans, it can be reasonably anticipated that it will exert a similar effect to that seen in rats, if we are dealing with the *ingestion* of ^{137}Cs . Whether this is valid for a *parenteral* contamination is not yet clear, since the enteral secretion rate in humans is not yet known. Although the ratio of faecal to urinary ^{137}Cs -excretion in humans is, by and large, comparable to the ratio in rats (Rosoff *et al.* 1963, Mraz and Patrick 1957 a), the *net* excretion is definitely lower. Experiments aiming at the elucidation of this question as well as on the elaboration of an optimal dosage and treatment schedule are urgently needed.

We wish to thank Miss H. Reckert and Miss D. Domprobst for skilful technical assistance. Sodium tetraphenylborate (Kalignost) was obtained by courtesy of Heye and Co., Berlin-Steglik.

SUMMARY

The effectiveness of several compounds in suppressing the enteral absorption of ^{137}Cs in rats was tested. All agents were found to be ineffective, with the exception of sodium tetraphenylborate, potassium ferrocyanide and, in particular, ferric ferrocyanide (Prussian Blue). The decrease in enteral absorption of ^{137}Cs by oral administration of PB is dependent upon dose and time of administration. The maximal effect achieved is the reduction of the body-burden by 99%. Repeated oral administration of PB also enhances substantially the excretion of parenterally administered ^{137}Cs . This effect was found to be independent on the time when treatment was started. No toxic side effects of PB were observed.

RÉSUMÉ

L'influence de Bleu de Prusse et d'autres composés sur la rétention de radio-césium par le rat.

On a étudié l'efficacité de plusieurs substances en ce qui concerne la suppression de l'absorption intestinale du ^{137}Cs chez le rat. La plupart des agents se sont révélés inefficaces à l'exception du tétraphénylborate de sodium, du ferrocyanate de potassium et en particulier du ferrocyanate ferrique (PB). Le PB, administré par voie orale, réduit l'absorption intestinale du ^{137}Cs en fonction de la dose et du temps. L'effet maximum obtenu a été une réduction de 99 pour cent de l'absorption. Des doses répétées réduisent aussi considérablement la rétention du ^{137}Cs injecté par voie parentérale. Cet effet ne dépend pas de l'intervalle de temps entre l'injection du ^{137}Cs et le traitement. Le PB n'a présenté aucun effet toxique secondaire.

ZUSAMMENFASSUNG

Die Einwirkung von Berlinerblau und anderen Verbindungen auf die Retention von Radiozäsium im Rattenkörper.

Es wurde der Einfluss verschiedener Substanzen auf die enterale Resorption von ^{137}Cs bei der Ratte untersucht. Alle Substanzen erwiesen sich als unwirksam mit Ausnahme von Natriumtetraphenylborat, Kaliumcyanoferrat (II) und vor allem von Ferricyanoferrat (II) (PB). Die Hemmung der enteralen ^{137}Cs -Resorption durch oral verabreichtes PB hängt von der Dosis und vom Zeitpunkt der Verabfolgung ab. Maximal wird eine Hemmung um 99% erreicht. Wiederholte orale Verabfolgung von PB bewirkt ebenfalls eine erhebliche Beschleunigung der Ausscheidung von intraperitoneal injiziertem ^{137}Cs , und zwar unabhängig vom Zeitpunkt der Behandlung. PB erwies sich als nichttoxisch.

Резюме

Влияние берлинской лазури и других соединений на удержание радиоактивного цезия в теле крысы.

Изучалось действие ряда веществ на резорбцию ^{137}Cs из кишечника крысы. Все вещества оказались неэффективными за исключением тетрафенилбората натрия, ферроцианида калия и, в особенности, ферроцианида железа (III) (PB). PB подавлял резорбцию ^{137}Cs в зависимости от дозы и от времени введения. Максимальное снижение резорбции составляет 99%. Повторное оральное введение PB также в значительной степени ускоряет выведение введенного парентерально ^{137}Cs . Этот эффект зависит от времени введения. PB не вызывает побочных токсических эффектов.

REFERENCES

- FATEYEVA, M. N., KLIMOV, V. S., PONIZOVSKAYA, A. I., GORBARENKO, N. I., SOKOLOV, V. V., and SMIRNOVA, M. I., 1960, *Mediz. Radiol.*, **5**, No. 7, 14.
- KURLANDSKAYA, E. B., 1957, *Mater. Toks. Radioakt. Vesh.*, **1**, 200.
- KYRSH, M., and ZVYAGINTSEV, O. E., 1958, *Zhur. Neorg. Khim.*, **3**, 2582.
- MCNEILL, K. G., GREEN, R. M., and RAPOPORT, A., 1962, Whole-Body Counting, Vienna, IAEA, p. 357.
- MOORE, W., and COMAR, C. L., 1962, *Int. J. Rad. Biol.*, **5**, 247
- MOSKALEV, YU. I., 1961 a, in "Raspredeleniye, Biologicheskoye Deystviye i Migratsiya Radioaktivnykh Isotopov". Moscow, Medgiz, p. 5.
- MOSKALEV, YU. I., 1961 b, *ibid.*, p. 141.
- MRAZ, F. R., and PATRICK, H., 1957 a, *Proc. Soc. expt. Biol. Med.*, **94**, 409.
- MRAZ, F. R., and PATRICK, H., 1957 b, *Arch. Biochem. Biophys.*, **71**, 121.
- NIGROVIĆ, V., 1963, *Int. J. Rad. Biol.*, **7**, 307.
- OGAWA, E., MACHIDA, J. I., SUZUKI, SH., and SHIBATA, K., 1958, *Gunma J. Med. Sci.*, **8**, 139.
- PUSHKAREV, V. V., SKRYLEV, L. D., and BAGRETSOV, V. F., 1960, *Zhur. Priklad. Khim.*, **33**, 81
- RICHMOND, C. R., and FURCHNER, J. E., 1961 a, *Health Physics*, **6**, 36.
- 1961 b, *Proc. Soc. expt. Biol. Med.*, **108**, 797.
- RICHMOND, C. R., FURCHNER, J. E., and LANGHAM, W. H., 1962, *Health Physics*, **8**, 201.
- ROGINSKIY, S. Z., MALININA, E. V., YANOVSKIY, M. I., ALTSHULER, O. V., and MOROKHOVETS, A. E., 1960, *Radiokhimiya*, **2**, 438
- ROSOFF, B., COHN, S. H., and SPENCER, H., 1963, *Rad. Research*, **19**, 643.
- TANANAYEV, I. V., GLUSHKOVA, M. A., and SEIFER, G. B., 1956, *Zhurn. neorg. Khim.*, **1**, 66.
- WASSERMAN, R. H., COMAR, C. L., and TAPPER, D. N., 1963, *Proc. Soc. expt. Biol. Med.*, **113**, 305.