Studies Concerning the Effect of Ferric Ferrocyanide, Beet Pulp, and Fluoride upon ^{137}Cesium Retention in the Rat (33696)

JOHN R. WOLSIEFFER, GEORGE K. STOOKEY, AND JOSEPH C. MUHLER
Department of Preventive Dentistry, Indiana University School of Dentistry, Indianapolis, Indiana 46202

Within the past 15 years, considerable effort has been devoted to investigations of the general metabolism of ^{137}Cs in various organisms. The practical importance of such studies has been stressed by many investigators concerning the potential hazard of ^{137}Cs, a by-product of nuclear fallout and peaceful applications of nuclear energy. ^{137}Cs is a high yield fission product of uranium and plutonium which exhibits a physical half-life of 30 years, and results in widespread contamination of the earth with particularly significant levels in certain areas such as the arctic regions. Man's acquisition of the isotope is largely by means of food as it is readily absorbed by plants and animal tissues. Due to the implications of both somatic and genetic hazards to man and animals, numerous approaches have been made in an attempt to accelerate the loss or decrease the biologic half-life of ^{137}Cs from the organism.

To date, various ferrocyanide compounds, ferric ferrocyanide (Prussian blue) in particular, appear to be the most promising thearapeutic agents reported in the literature (1–9). Since ferric ferrocyanide acts by binding ^{137}Cs entering or being secreted into the intestinal tract, and hence prevents its absorption or reabsorption, it was felt that if one or more agents could be found to act synergistically with ferric ferrocyanide to increase urinary excretion of ^{137}Cs, to enchance the rate of endogeneous secretion of ^{137}Cs into the intestine, and/or to selectively bind ^{137}Cs in the intestine, the effectiveness of the system would be even greater. Also, it is conceivable that with a synergistic system a lower concentration of a given agent may suffice in order to attain the desired physiological effect.

Williams and Patrick (10) have demonstrated in rabbits given ^{137}Cs intraperitoneally that dietary beet pulp produces a significant increase in fecal ^{137}Cs loss, although it did not increase significantly total ^{137}Cs excretion. It was felt, however, that this increase in intestinal ^{137}Cs coupled with the action of the ferric ferrocyanide might produce a significant synergistic effect. In our unpublished work, fluoride has shown some indication of increasing ^{137}Cs excretion in the rat.

This study was conducted, therefore, in an attempt to investigate the possible synergistic effects of various combinations of ferric ferrocyanide, beet pulp, and fluoride upon the elimination of radiocesium (^{137}Cs) in the rat.

Materials and Methods. A total of 96 male rats of the Wistar strain[1] 30 days of age (av wt. 58.8 g) was divided (according to their initial body wt.) into 8 equal groups. All animals were housed individually in raised screen cages in an air-conditioned room, were weighed at 15-day intervals throughout the duration of the experiment, and received a stock corn diet[2] and distilled water *ad libitum*. At 45 days of age, the rats of Groups A and B (controls) continued to receive the same stock corn diet, whereas the rats of Groups C through H for the remainder of the experiment were given the stock corn diet modified by the substitution of ferric ferrocyanide, beet pulp, and/or fluoride for an equal amount of yellow corn meal. The experimental groups, received the following regimens: Group C, 1.0% ferric ferrocyanide; Group D, 20.0% ground beet pulp; Group E, 0.007% NaF; Group F, 1.0% ferric ferrocyanide plus 20.0% ground beet plup; Group G, 1.0% ferric ferrocyanide plus 0.007% NaF; and Group H, 1.0% ferric ferrocyanide

[1] Harlan Industries, Cumberland Indiana.

[2] Percentage composition of this diet was as follows: yellow corn meal, 64.0; powdered whole milk, 30.0; alfalfa meal, 4.8; irradiated yeast, 0.2; and iodized salt, 1.0.

TABLE I. Modifications in Dietary Constituents of Stock Corn Diet (expressed as % of diet).[a]

Group	Yellow corn meal	Prussian blue	Ground beet pulp	Sodium fluoride
A	64.0	—	—	—
B	64.0	—	—	—
C	63.0	1.0	—	—
D	44.0	—	20.0	—
E	64.0	—	—	0.007
F	43.0	1.0	20.0	—
G	63.0	1.0	—	0.007
H	43.0	1.0	20.0	0.007

[a] Unmodified dietary constituents not shown.

plus 20.0% ground beet pulp plus 0.007% NaF. The composition of each of these regimens is summarized in Table I.

At 60 days of age, following a 15-day dietary acclimatization period, each rat of Groups B through H received 40 μCi of carrier-free ^{137}Cs intrapertioneally.[3] After 30 days all the animals were sacrificed by chloroform inhalation with muscle, femur, and remaining carcass tissues surgically removed and analyzed for ^{137}Cs. The muscle tissues were individually dried under infrared lamps, vacuum desiccated to a constant weight, and wet-ashed with nitric acid. The femurs and remaining carcasses were individually ashed in silica dishes for 6 hr at 600°. Nitric acid solutions of all samples were plated on stainless steel planchets, dried, and counted by a scintillation detector equipped with an end-window Geiger-Muller tube. Computations and analysis of variance were carried out by means of an IBM 7040 Computer. The Bartlett chi-square test for homogeneity of variance was used and, in cases in which homogeneity of variance could not be assumed, the Welch test modified this variable. Tukey's w procedure was used to test for significant differences between individual group means.

Table II shows the mean weights of the respective groups at successive 15-day intervals and the mean weight gain for each group. From an analysis of variance, it was inferred that at a 5% level of significance there was no significant difference in the mean weights of the different groups at any given time interval. Therefore, on the basis of these data, the various dietary regimens did not significantly alter the growth rate of the animals.

TABLE II. Summary of Growth and Mortality Data.

Group	Regimen	No. of animals		Mean wt.[a]					
		Initial	Final	Initial	15 days	30 days	45 days	60 days	Mean wt. gain
A	Control	12	12	58 ± 3	112 ± 6	180 ± 9	255 ± 9	323 ± 11	265 ± 9
B	Control	12	12	58 ± 2	111 ± 6	181 ± 7	240 ± 9	310 ± 9	252 ± 8
C	Prussian blue (P)	12	12	58 ± 2	112 ± 5	189 ± 9	261 ± 9	324 ± 10	266 ± 9
D	Beet pulp (B)	12	12	58 ± 2	112 ± 5	183 ± 9	258 ± 11	319 ± 12	260 ± 11
E	Fluoride (F)	12	12	59 ± 2	112 ± 5	178 ± 4	251 ± 5	321 ± 10	263 ± 10
F	(P + B)	12	12	62 ± 3	112 ± 5	184 ± 7	267 ± 10	331 ± 11	269 ± 10
G	(P + F)	12	12	58 ± 3	112 ± 5	179 ± 5	256 ± 7	313 ± 9	255 ± 9
H	(P + B + F)	12	12	58 ± 2	112 ± 5	190 ± 8	268 ± 7	336 ± 8	278 ± 7
F value from analysis of variance[b]				0.2943	0.0000	0.3498	1.3065	0.7279	0.7549

[a] Expressed as g of body wt. ± SE of the mean.
[b] Critical region $F > 2.8486$ (5% confidence level).

Table III shows the effects of the respective dietary regimens upon ^{137}Cs retention 30 days following the single intraperitoneal injections of the isotope. All data are expressed as percentage of injected dose per gram. However, the values for muscle data are expressed as percentage of injected dose per

[3] The rats of Group A served as controls and received no ^{137}Cs; those in Group B served as a positive control and received the ^{137}Cs and the control diet.

TABLE III. ^{137}Cs Retention 30 Days Following a Single Intraperitoneal Injection of 40 μCi of ^{137}Cs.

Group	Regimen	% Injected dose/g ($\times 10^{-3}$)[a]		
		Muscle[b]	Femur[c]	Carcass[c]
B	Control	93.8 ± 5.6	23.2 ± 2.6	310. ± 53
C	Prussian blue (P)	3.63 ± 0.45	0.854 ± 0.14	18.5 ± 6.1
D	Beet pulp (B)	42.2 ± 4.0	27.7 ± 2.5	196. ± 49
E	Fluoride (F)	79.9 ± 5.7	3.15 ± 0.42	166. ± 48
F	(P + B)	1.33 ± 0.24	0.694 ± 0.192	0.884 ± 0.742
G	(P + F)	3.95 ± 0.44	2.40 ± 0.46	0.053 ± 0.053
H	(P + B + F)	1.25 ± 0.19	0.704 ± 0.164	0.000 ± 0.000

[a] Expressed as mean value ± SE of the mean.
[b] % Injected dose/g of tissue (wet wt.) $\times 10^{-3}$.
[c] % Injection dose/g of ash (ash wt.) $\times 10^{-3}$.

gram of tissue (wet wt.), whereas, those for femur and carcass data are expressed as percentage of injected dose per gram of ash weight.

The muscle of the control animals contained 93.8 $\times 10^{-3}$% of the injected dosage of ^{137}Cs whereas the animals provided ferric ferrocyanide (Group C) had only 3.63 $\times 10^{-3}$%. This latter value is 96.1% less than that of the control animals. The tissues of the animals provided beet pulp (Group D) and fluoride (Group E) contained 42.2 $\times 10^{-3}$ and 79.9 $\times 10^{-3}$% of the injected dose of ^{137}Cs representing reductions of 55.0 and 14.8%, respectively. The various combinations of ferric ferrocyanide plus beet pulp, ferric ferrocyanide plus fluoride, and ferric ferrocyanide plus beet pulp plus fluoride resulted in the elimination of 98.6, 95.7, and 98.7% of the injected ^{137}Cs, respectively.

The femur ^{137}cesium retention data indicate that the ingestion of ferric ferrocyanide resulted in the elimination of 96.5% of the isotope. The ingestion of beet pulp had no effect upon the elimination of the element while the ingestion of fluoride resulted in a significant elimination of 86.7% of the element. All of the combination regimens (Groups F–H) resulted in more than 89.7% elimination of the element.

The carcass ^{137}Cs retention data indicate that all of the regimens were effective in the elimination of the isotope from the body. With the exception of the fluoride-containing regimens the trends in effectiveness of the various regimens closely paralleled those found in muscle. The trends with the fluoride-containing regimens more closely followed the findings in the femurs. The use of fluoride alone resulted in an elimination of 46.5% while the combination of fluoride with ferric ferrocyanide and beet pulp resulted in the complete removal of the isotope from the body. Analysis of variance for each set of data in Table III shows a significant F$^-$ ratio.

Discussion. Based upon the statistical tests at both 5 and 1% levels of significance, the ferric ferrocyanide (Prussian blue) showed a significant effect in reducing ^{137}Cs retention in each of the examined tissues, whereas, beet pulp showed a significant effect only in muscle tissue. Fluoride had a significant effect only in femur tissue. Each of the possible synergistic systems (Groups F, G, and H) when compared with the control group (Group B) showed a significant effect in reducing ^{137}Cs retention in each of the tissues. The addition of beet pulp to ferric ferrocyanide showed a significant ($p<0.05$) reduction in ^{137}Cs retention in muscle tissue but no significant change in ^{137}Cs retention in the femur. It seems probable that there may be a reduction of ^{137}Cs in the carcass although the t value in this case is slightly less than its corresponding critical level. In the case of the carcasses of Groups F, G, and H, activity measurements were so low that accuracy of measurement was difficult and hence the corresponding variances were relatively high

which may account for the t values being lower than expected. The addition of fluoride to ferric ferrocyanide showed no significant change at the 5% level of significance in ^{137}Cs retention in any of the examined tissues although the t values of the femur and carcass data are close to their corresponding critical values. The addition of both beet pulp and fluoride to ferric ferrocyanide showed a significant ($p<0.05$) reduction of ^{137}Cs retention in muscle tissue but no significant changes in ^{137}Cs retention in the femur. A significant change in ^{137}Cs retention in the carcasses from the animals on this latter regimen due to the addition of fluoride and beet pulp separately to the ferric ferrocyanide as compared to ferric ferrocyanide alone is not indicated by the test, but again the t value is near the critical value. The data involving the addition of both beet pulp and fluoride to ferric ferrocyanide is in agreement with the combined effects of the separate additions of these materials to the ferric ferrocyanide.

As might be expected, the effects of the various dietary regimens upon the retention of ^{137}Cs in the carcasses appear to be intermediate to that of muscle and femur. It should be noted that due to the low ^{137}Cs activity of the carcasses of Groups F, G, and H, caution should be used in the interpretation of the additive effects observed.

Due to the conservative nature of Tukey's w test, additional statistical comparisons would probably appear significant should a less conservative test be used. Such analyses have been made and will be provided upon request.

The results of this study confirm previous reports of the value of ferric ferrocyanide in the partial elimination of ^{137}cesium from biological systems. The finding of complete elimination of the isotope from the carcasses of the animals provided a combination of fluoride, ferric ferrocyanide, and beet pulp is of interest and requires additional study. The significant effect of fluoride observed in the elimination of ^{137}Cs from the femur suggests the need for additional investigations concerning the amount of fluoride dosage and exposure required to provide optimal elimination as well as an investigation of the comparative value of various fluoride salts since biological variations in the metabolism of different fluorides are known.

Summary. The individual and additive effects upon the elimination of ^{137}cesium from the body of supplementation of the diet with 1% ferric ferrocyanide (Prussian blue), 20% beet pulp, and 0.007% sodium fluoride was determined in rat muscle, femur, and carcass 30 days following a single intraperitoneal injection of the isotope. Ferric ferrocyanide showed a significant effect in reducing ^{137}Cs retention in each of the examined tissues. Beet pulp showed a significant effect in reducing ^{137}Cs retention in muscle tissue when used separately, and a greater effect when used in combination with ferric ferrocyanide and fluoride. Fluoride significantly reduced ^{137}Cs retention in the femur when used separately. The combination of all dietary regimens resulted in the removal of 98.7 and 97.0% of the ^{137}Cs in muscle and femur, respectively, and in the complete elimination of ^{137}Cs from the remaining carcass of the rat.

The authors are grateful to Mrs. Adeline C. Seifert of the IUMC Research Computation Center, which is supported in part by Public Health Service Research Grant Fr 00162, for her assistance in the computer programming for the statistical analysis.

1. Brenot, A., Chiadot, P., and Rinaldi, R., Ann. Biol. Clin. (Paris) **24**, 1223 (1966).

2. Brenot, A. and Rinaldi, R., Pathol. Biol. **15**, 55 (1967).

3. Havlicek, F., Kleisner, I., Dvorak, P., and Pospisil, J., Strahlentherapie **134**, 123 (1967).

4. Madshus, K., Stromme, A., Bohne, F., and Nigrovic, V., Intern. J. Radiation Biol. **10**, 519 (1966).

5. Nigrovic, V., Intern. J. Radiation Biol. **1**, 307 (1963).

6. Nigrovic, V., Phys. Med. Biol. **10**, 81 (1965).

7. Nigrovic, V., Bohne, F., and Madshus, K., Strahlentherapie **130**, 413 (1966).

8. Richmond, C. R., U. S. At. Energy Comm. Rept. LA-DC-8473, (1967).

9. Richmond, C. R. and Bunde, D. E., Proc. Soc. Exptl. Biol. Med. **121**, 664 (1966).

10. Williams, L. G. and Patrick, H., Arch. Biochem. Biophys. **70**, 464 (1957).

Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.