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COMPARATIVE METABOLISM OF RADIONUCLIDES IN MAMMALS. III.

RETENTION OF ⁵⁴MANGANESE BY FOUR MAMMALIAN SPECIES*

54 Mn

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ABSTRACT--Radiomanganese (^{54}Mn) was administered to mice and rats by the oral, intravenous, and intraperitoneal routes and to dogs and monkeys by the oral and intravenous routes. Whole-body counting techniques, utilizing 4π liquid scintillation counters, were used to determine whole-body retention parameters. Retention functions consisting of the sum of three or four exponential expressions were adequate descriptions of retention until the body burden was less than 1 percent of the administered dose. The longest effective half-times after intravenous injections were 119, 146, 99, and 68 days for mice, rats, monkeys, and dogs, respectively. Tissue distribution studies in rats showed that, for most tissues, concentration as a function of time roughly paralleled whole-body retention. Both bone and brain were found to have a slower rate of loss than other tissues. Using a parabolic relation between body weights and the integrals of the retention functions, it was estimated that $7 \times 10^{-3} \frac{\mu\text{Ci}}{\text{g}}$ was the maximum permissible concentration in water for man. This value is in good agreement with the current ICRP recommended value of 8×10^{-3} .

INTRODUCTION

Although the role of manganese as an essential trace element has been widely investigated, "The biology of manganese suffers from the lack of unifying principles."⁽¹⁾ Several authors attribute a role in oxidative phosphorylation,^(2,3) fatty acid,⁽⁴⁾ and cholesterol synthesis.⁽⁵⁾ Other functions are also postulated.^(1,6,7) Although no isotopes of manganese are found in fission products, ^{54}Mn has been found in fallout, presumably an activation product formed during weapons tests.⁽⁸⁻¹⁰⁾ Although the presence of ^{54}Mn in fallout presents no great health hazard,⁽¹⁰⁾ interest in relevant retention parameters is stimulated by its appearance. The extensive work on manganese furnishes a relatively small amount of retention data. The following study was executed to remedy the deficit.

METHODS

The animals were RF female mice, Sprague-Dawley male rats, beagle hounds, and Macaca mulatta monkeys. All were maintained on standard commercial diets and tap water. Excreta samples were collected periodically for assay of ^{54}Mn activity. The ages, weights, number of animals used, route of administration, amount administered, and the duration of the counting periods are given in Table 1. The ^{54}Mn activity in mice and rats was assayed in LASAC III, a 4π liquid scintillation gamma-ray detector.⁽¹²⁾ For dogs and monkeys a similar but larger detector (HUMCO II) was used.⁽¹³⁾ The data processing

procedure has been described. (14) A group of 44 rats was injected intraperitoneally with ^{54}Mn . The animals were then killed by ether anesthesia in groups of 4 to determine the tissue distribution as a function of time. The following tissues were assayed for ^{54}Mn activity: pelt, gut (esophagus to rectum, with contents), liver, kidneys, spleen, lungs, heart, testes, brain, blood, muscle, and bone. The carcass (whole body minus pelt and viscera) and the remains (viscera, ^{not mentioned above,} mesentery, fat, and blood) were also assayed.

Because 2 rats in the oral group (I) had whole-body activities more than a factor of 10 greater than the remaining animals in the group, a second group of 6 oral rats (II) was assayed for a like period. One rat in this group showed a retention pattern similar to the "high" rats in oral group I.

RESULTS

Results are presented as effective retention (no correction for physical decay). The data have been fit to the sum of 3 or more exponentials. Intercept parameters, given by the computer in counts per second, have been converted to percentages. Data from individual animals were processed to determine the "best-fit," and data from all animals in a group were processed to determine the best-fit to the group's data. Table 2 gives retention parameters for rats injected with ^{54}Mn intraperitoneally. The best-fit to the

group values is given in the first row (labeled 1-6). The next 6 rows give the same values for individuals, while the last row gives means of values for individual rats. The last column presents an estimate of the equilibrium level for conditions of chronic exposure. In Table 3 retention parameters for each group are given; that is, the retention parameters represent the best-fit to all whole-body retention measurements made on all individuals in each group.

In Fig. 1 the best-fit expressions describing retention in mice after IV, IP, and oral administration are plotted (smooth curves) along with an indication of average values (IV, open circles; IP, closed circles; and oral, closed triangles) and ranges of values (vertical bars). It should be understood that the values presented in Fig. 1 are representative, approximately every 20th day being shown; the omissions are made for the sake of clarity. In Fig. 2 the smooth curves are plots of the first derivatives of the retention functions. The data points represent the excretion rates at various times after administration.

Figures 3 and 4 present similar data for the rats, with the addition of "high" oral rats in Fig. 3. This line represents retention by the 3 rats (2 from group I, 1 from group II) which were distinctly different from the remaining oral rats, which are represented by the smooth curve labeled oral "normal." The vertical bars and lines again represent ranges, not deviations. In Fig. 4 the smooth curve for the excretion rate data for the orally administered ^{54}Mn is a plot of the derivative of a weighted average of the retention parameters

of the "high" and "normal" rats, along with the excretion data of oral groups I and II. The other smooth curves are plots of the derivatives of the retention functions for IV and IP rats, along with the respective excretion rate measurements.

Figures 5 and 6 present data for dogs and monkeys. Again, only data for about every 20th day are presented for the sake of clarity. The circles and squares represent average values, while the vertical lines indicate the range of values.

Concentration in rat tissue as a function of time is presented in Figs. 7 and 8. The smooth curve through the "whole-body" symbols is a plot of the best-fit retention function for IP rats. The symbols are average whole-body retention values for the 4 rats at each point. The ordinate scale for this line and these points is indicated on the right border of the illustration.

DISCUSSION

It seems apparent that small differences in whole-body retention result when manganese is given by the IP or IV route. In both rats and mice the IV manganese is lost more slowly than IP manganese (Figs. 1 and 3). Kent and McCance⁽¹⁵⁾ imply that differences due to route of administration may occur. Greenberg et al.⁽¹⁶⁾ dispute this, but their measurements extend only to 48 hours. The differences certainly are small (less than 1 percent of the administered dose at 200 days for both rats and mice) and undetectable at early times or by excreta assay.

The agreement between the plots of the first derivatives of the retention function and the excretion rate data points for each group indicates that weight changes in the animals had little or no effect on counting rates and that excreta were collected with a fair degree of efficiency. This efficiency was due to the almost completely fecal route of excretion. The urinary-fecal ratios were always less than 1.0 and seldom as large as 0.3. The remarkable "outliers" among the oral rats are unexplained. The fact that these 3 animals appeared on two separate occasions (2 in group I oral rats and 1 in group II oral rats) and fell into and remained in a close group argues for some real physiological variable. The similarity to the IP data suggests the possibility of a punctured stomach, but there were no mortalities for more than 4 months. Rats maintained on ⁵⁴Mn-contaminated drinking water will be compared with rats given occasional gastric gavages in order to determine if the means of administration is a causal factor.

Retention in tissues seems to be much like whole-body retention except for brain and bone. Both of these tissues indicate a slower turnover time, and brain gives a strong suggestion of an impeded early uptake (Fig. 7). This may well be a reflection of a blood-brain barrier⁽¹⁷⁾ against the passage of the metal. Fore and Morton⁽¹⁸⁾ report that the highest concentrations are found in bone in a number of reports on various species. However, Tipton (cited in Ref. 11) finds only a low concentration in human bone.

It is obvious that (except for 3 anomalous rats) little manganese

is absorbed from the gut (oral groups in Figs. 1, 3, 5, ~~and 9~~ and in Table 3). The rapid passage through the gut soon reduces the whole-body burden to levels where the accuracy of counting is marginal without prolonged counting times. The curtailed duration of the experiment due to this factor may be compensated for by use of the parameters from the IV administration (see Ref. 19 for an explanation of this practice). The broken lines in Figs. 1 and 5 show the result of such extensions to the oral data. The parameters labeled "adjusted oral" in Table 3 were used to plot these lines.

The integrals (last column in Table 3) of the adjusted oral retention functions are plotted as a function of body weight and fit to a parabolic function (Fig. 9) which may be used to estimate the integral for man at 70 kg. (19-21) Because the integral of the effective retention function affords an estimate of the equilibrium level (in terms of the daily dose) for a chronic exposure to a constant dose level, the value may be used to estimate MPC values. (19, 20) If we consider the whole body to be the target organ (a not unreasonable assumption in view of the relatively uniform distribution and energy of ^{54}Mn), then $\text{MPC}_w = \frac{q}{E S}$, where q is the maximum permissible body burden (40 μC), (11) E is the estimated equilibrium level (2.6 times the daily dose, Fig. 9), and S is the daily water intake (2.2×10^3 ml), will give an estimate of the maximum permissible concentration of ^{54}Mn in water for continuous daily exposure. A value of 7×10^{-3} $\mu\text{Ci/ml}$ is obtained from substitution of the above values. The ICRP value is 8×10^{-3} . (11)

The value of 7×10^{-3} $\mu\text{Ci/ml}$ was obviously obtained without reference to human parameters. Cotzias et al. ⁽²²⁾ present some illustrations (but no ^{values} ~~figures~~) which may be used to estimate an equilibrium level for humans. Half-times of ~ 65 and 240 days after IV injection were estimated from Figs. 7 and 8. ⁽²²⁾ If we assume that the human retention will be partitioned similarly to the retention in monkeys, an equilibrium level of about 3.6 is found. Such a level would make the $\text{MPC}_w = 5 \times 10^{-3}$ $\mu\text{Ci/ml}$. The difference from the ICRP value is small and, in view of the uncertainties involved, is a crude confirmation of the ICRP value.

Table 1. Protocol for administration of $^{54}\text{MnCl}_2$ to four species of mammals

Species	Route of Administration	No. of Animals	Age (months)	Weight (kg)	Administered Dose (μCi)	Days to Last Count
Mice	IV	12	2.95	0.0233	0.53	259
Mice	IP	12	2.63	0.0236	0.44	252
Mice	Oral	12	2.63	0.0245	0.44	14
Rats	IV	6	2.90	0.3270	0.44	217
Rats	IP	6	2.90	0.3440	0.50	217
Rats	Oral I	6	2.00	0.3370	0.50	135
Rats	Oral II	6	2.90	0.3300	0.44	135
Rats	IP	44	2.90	0.3250	0.53	89
Dogs	IV	4	90	13.1	0.60	423
Dogs	Oral	4	91	16.5	0.60	46
Monkeys	IV	3	~36	8.46	0.60	290
Monkeys	Oral	3	~36	6.85	0.60	23

Table 2. Effective retention parameters in rats after intraperitoneal injection of $^{54}\text{MnCl}_2$

	a_1^*	k_1^*	$T_{1/2}$	a_2	k_2	$T_{1/2}$	a_3	k_3	$T_{1/2}$	E.F. **
1-6	54.16	0.1176	5.9	41.92	0.0377	18.4	3.92	0.0072	96.4	21.2
1	57.89	0.1243	5.6	38.14	0.0374	18.5	3.96	0.0077	89.7	20.0
2	59.73	0.0950	7.3	37.58	0.0340	20.4	2.69	0.0059	117.9	21.9
3	42.31	0.1336	5.2	53.51	0.0404	17.2	4.18	0.0076	91.7	21.9
4	63.78	0.1013	6.8	34.58	0.0315	22.0	1.64	0.0041	168.9	21.3
5	48.56	0.1455	4.8	46.02	0.0465	14.9	5.43	0.0096	72.3	18.9
6	54.59	0.1178	5.9	39.82	0.0356	19.5	5.59	0.0064	108.0	24.5
$\bar{X}$	54.48	0.1196	5.9	41.61	0.0376	18.8	3.92	0.0069	108.1	21.4

$$^*\text{Retention} = R_t = \sum_{i=1}^n a_i e^{-k_i t}.$$

$$^{**}\text{Equilibrium Factor} = \int_0^{\infty} R_t dt.$$

Table 3. Retention parameters for ^{54}Mn in mice, rats, dogs, and monkeys

Species	Route of Admin.	No. of Animals	a_1^*	k_1^*	a_2	k_2	a_3	k_3	a_4	k_4	E.F. **
Mice	IV	12	16.18	1.501 (0.46)***	39.07	0.144 (4.8)	38.38	0.0291 (24)	6.37	0.00581 (119)	27.0
Mice	IP	12	26.23	0.332 (2.1)	40.49	0.093 (7.5)	29.83	0.0268 (26)	3.44	0.00549 (126)	22.6
Mice	Oral	12	95.91	4.780 (0.14)	3.70	1.040 (0.67)	0.39	0.0349 (19)	--	--	0.35
Mice	Oral adjusted	12	95.83	4.780 (0.14)	3.77	1.053 (1.66)	0.27	0.0601 (11)	0.13	0.00581 (126)	0.51
Rats	IV	6	12.89	1.039 (0.67)	46.36	0.126 (5.5)	36.13	0.0309 (22)	4.62	0.00474 (146)	25.2
Rats	IP	6	--	--	54.16	0.118 (5.9)	41.92	0.0377 (18)	3.92	0.00719 (96)	21.2
Rats	Oral "high"	3	70.97	1.987 (0.35)	--	--	22.50	0.0474 (15)	6.53	0.01210 (57)	10.5
Rats	Oral "normal"	9	98.09	2.550 (0.27)	1.47	0.114 (6.1)	0.44	0.0145 (48)	--	--	0.82
Rats	Oral adjusted	9	97.18	2.330 (0.30)	1.93	0.231 (3.0)	0.76	0.0381 (18)	0.12	0.00474 (146)	0.95
Monkeys	IV	3	13.20	0.298 (2.3)	39.80	0.0375 (18)	47.00	0.00698 (99)	--	--	78.4

Table 3 (continued)

Species	Route of Admin.	No. of Animals	a_1	k_1	a_2	k_2	a_3	k_3	a_4	k_4	E.F.
Monkeys	Oral	3	95.39	2.283 (0.30)	3.91	0.2950 (2.3)	0.70	0.01220 (58)	--	--	1.13
Monkeys	Oral adjusted	3	97.22	2.067 (0.34)	2.26	0.1870 (3.7)	0.52	0.00698 (99)	--	--	1.33
Dogs	IV	4	14.78	0.223 (3.1)	52.53	0.0381 (18)	32.69	0.0102 (68)	--	--	46.4
Dogs	Oral	4	92.54	4.155 (0.17)	3.02	0.1920 (3.6)	4.45	0.0337 (21)	--	--	1.70
Dogs	Oral adjusted	4	92.11	4.172 (0.17)	1.95	0.3820 (1.8)	4.68	0.0636 (11)	1.26	0.0102 (68)	2.24

*Retention = $R_t = \sum_{i=1}^n a_i e^{-k_i t}$, where a_i and k_i are the constants given in this table, e is the base of natural logs, and t is the time in days.

**Equilibrium Factor = $\int_0^{\infty} R_t dt$.

***Values in parentheses are effective half-times in days.

REFERENCES

1. G. C. COTZIAS, Mineral Metabolism, Vol. 2, Part B (C. L. Comar and F. Bronner, eds.), Academic Press, Inc., N. Y. (1962), pp. 403-442.
2. O. LINDBERG and L. ERNSTER, Nature 173, 1038 (1954).
3. J. R. BRONK and W. W. KIELLY, Federation Proc. 16, 158 (1957).
4. M. O. AMDUR, L. C. NORRIS and G. F. HEUSER, J. Biol. Chem. 164, 783 (1946).
5. G. L. CURRAN, Proc. Soc. Exptl. Biol. Med. 88, 101 (1955).
6. W. F. VON OETTINGEN, Physiol. Rev. 15, 175 (1935).
7. G. C. COTZIAS, Physiol. Rev. 38, 503 (1958).
8. W. H. SHIPMAN, P. SIMONE and H. V. WEISS, Science 126, 971 (1957).
9. P. F. GUSTAFSON, S. E. MUNIAK and S. S. BRAR, Nature 203, 470 (1964).
10. F. SHAFFER and F. LUDWIG, Atompraxis 9, 75 (1963).
11. K. Z. MORGAN, Health Phys. 3 (1960).
12. C. R. RICHMOND, J. E. LONDON, J. S. FINDLAY and J. E. FURCHNER, Los Alamos Scientific Laboratory Report LA-3132-MS (1964), pp. 76-83.
13. M. A. VAN DILLA and E. C. ANDERSON, Whole-Body Counting, IAEA, Vienna (1962), pp. 41-58.
14. C. R. RICHMOND, J. E. FURCHNER, P. N. DEAN and P. McWILLIAMS, Health Phys. 10, 3 (1964).

15. N. L. KENT and R. A. McCANCE, Biochem. J. 35, 877 (1941).
16. D. M. GREENBERG, D. H. COPP and E. M. CUTHBERTSON, J. Biol. Chem. 147, 749 (1942).
17. L. S. MAYNARD and G. C. COTZIAS, J. Biol. Chem. 214, 489 (1955).
18. H. FORE and R. A. MORTON, Biochem. J. 51, 600 (1952).
19. C. R. RICHMOND, J. E. FURCHNER, G. A. TRAFTON and W. H. LANGHAM, Health Phys. 8, 481 (1962).
20. J. E. FURCHNER and C. R. RICHMOND, Health Phys. 9, 277 (1963).
21. C. R. RICHMOND, W. H. LANGHAM and T. T. TRUJILLO, J. Cell. Comp. Physiol. 59, 45 (1962).
22. G. C. COTZIAS, D. C. BORG and A. J. BERTINCHAMPS, Metal Binding in Medicine (M. Seven, ed.), Lippincott, Philadelphia (1960), pp. 50-58.

Fig. 1. Whole-body effective retention in mice after IV, IP, and oral administration. The data points are average values. The vertical bars represent the range of values. The smooth curves are plots of the retention functions.

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Fig. 2. Excretion rates of ^{54}Mn after IV and IP administration to mice. The data points represent the sum of the percentage activity found in urine and feces. The smooth curves are a plot of the first derivatives of the retention functions.

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Fig. 3. Whole-body effective retention in rats after oral, IV, and IP administration. The smooth curves are plots of the retention functions. The vertical lines and rectangles indicate ranges of values. The majority of the data points used to determine the retention are omitted for the sake of clarity.

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Fig. 4. Excretion rates of ^{54}Mn in rats. The data points are the percentage activity found in urine and feces. The smooth curve for excretion after oral administration is the first derivative of a retention function obtained from a weighted average of the retention functions of both "high" and "normal" oral rats.

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Fig. 5. Retention of oral and IV ^{54}Mn in dogs and monkeys.

The broken portion of the oral curve for dogs parallels the terminal portion of the IV curve for dogs. The same artifice has been used on the monkey oral curve.

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Fig. 6. Excretion of ^{54}Mn in dogs and monkeys after IV administration.

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Fig. 7. Tissue concentration of ^{54}Mn in rats. Both brain and bone concentrations decrease more slowly than other tissues.

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Fig. 8. Tissue concentrations of ^{54}Mn in rats. The whole-body effective retention function is plotted (smooth curve on top) with the average whole-body retention of each group at sacrifice. The scale for this curve is on the right. Concentration changes in the tissues parallel the whole-body retention.

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Fig. 9. The data points are the integrals of the retention functions (between zero and infinity) plotted as a function of body weight. The smooth curve is the least-squares fit to these points:
 $E.F. = 0.2205 (\text{Body Weight})^{0.2286}.$

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