

MANGANESE METABOLISM IN MAN: RAPID EXCHANGE OF Mn^{56} WITH TISSUE AS DEMONSTRATED BY BLOOD CLEARANCE AND LIVER UPTAKE ¹

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Despite the well-known physiological importance of manganese (1-7) and its implication in some disease states (2, 8-10), the exact nature of its metabolic role remains undefined (11, 12). In mammals manganese concentrates in organs rich in mitochondria, notably the liver (2-7, 11, 13, 14); and previous work from this group (11) has shown these organelles to be the major pool of radiomanganese turnover shortly after injection into animals. *In vitro* traces of the element have widespread biochemical activity (2, 3, 15-19), especially in relationship to mitochondria (20) and those of their enzyme systems that carry out oxidative phosphorylation (16, 21-26).

The present study of blood clearance and of radiation measured at the body surface following Mn^{56} injection in man is an attempt to describe and to determine the kinetics of this turnover, which appears to correlate with mitochondrial function. From the analysis of these data in relation to the work of others it is also possible to gain insight into the mechanisms of transport directly from the blood. Our results further suggest that most of the normal body manganese is readily exchangeable and is not bound in metalloproteins or other highly stable configurations.

EXPERIMENTAL

Radioisotopic tracer. Mn^{56} (half-life: 2.58 hr.) ² was generated in the Brookhaven research reactor. One ml. aliquots containing 11.1 μ g. of $MnSO_4 \cdot H_2O$ (3.6 μ g. of Mn^{++}) in saline and representing 15 to 20 microcuries

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² The radioemission spectrum of Mn^{56} is complex, but the decay scheme includes several energetic gamma rays (most prominent at 845 Kev) (27) of such penetrating power that correction for self-absorption of radiation emanating from within the body was unnecessary. Bremsstrahlung associated with abundant high-energy negatrons provides a further source of externally detectable radiations.

of Mn^{56} ³ were injected into patients. The radiation decay of other aliquots was followed for 48 to 72 hours with a well-type NaI(Tl) scintillating crystal and scaling circuit. The resultant activity curves indicated the only contaminating radioelement to be a small amount of Na^{24} (about 0.5 per cent of the Mn^{56} activity at injection time), which was ignored in the subsequent treatment of the data.

Route of administration and withdrawal of blood samples. Following an overnight fast, patients received Mn^{56} tracer intravenously. Thereafter serial venous blood samples of approximately 3 ml. volume were withdrawn from the contralateral arm. Samples were promptly assayed for radioactivity and weighed. Corrections for radioactive decay were then made. Frequent samples were obtained during the first few minutes after injection, but later sampling frequency was decreased gradually to intervals of approximately five minutes.

Surface counting technique. Concurrently with the withdrawal of blood samples, radiation was monitored over the body surface with collimated detectors whose outputs were transcribed automatically.⁴ As anticipated from animal data (2, 4-7, 11), preliminary scanning following Mn^{56} injection revealed localization of the greatest radioactivity in the region of the liver. Accordingly, one scanner was positioned on the skin surface over the liver area, usually about 3 cm. above the costal margin in the right midclavicular line ("liver area" counter). A second counter was located over the right femur about 18 cm. above the patella ("thigh" counter). This was taken to be a "reference area" that would not reflect active visceral metabolism or selective manganese uptake.

The exact distribution of Mn^{56} *in situ* was unknown. Thus absolute calibration of the body surface counters with laboratory standards was impossible. Furthermore, two scanners of different sensitivity were used. Empirically, the response of one specific counter in a fixed position and orientation was taken as standard. The two scanners then were intercalibrated at late times, when the levels of external radioactivity had reached virtual equilibrium.

Standardized units of radioactivity. Intercomparison

³ This presented to the recipient a total-body radiation dose of less than 4 m-rad (28).

⁴ Voltage signals from conventional scaling circuits were recorded on separate channels of a standard polygraph, and the resulting frequency record was integrated manually and plotted as an amplitude curve (*cf.* Figure 1).

TABLE I
Patient data

Experiment	Date	Patient	Unit no.	Sex	Age	Major diagnosis
1	11/21/55	F. McD.	7090R	M	60	Cancer with metastases (some to liver)
2	11/22/55	(F. McD.)*				
3	12/19/55	R. E.	7107R	F	40	Wilm's tumor with metastases (some to liver)
4	3/30/56	F. M.	7275R	M	42	Cancer with metastases (none apparent in liver)
5	5/24/56	M. M.	7376R	M	65	Paralysis agitans—? idiopathic
6	6/8/56	A. B.	7343R	F	60	Paralysis agitans—postencephalitic; diabetes
7	7/26/56	J. McK.	7284R	F	42	Paralysis agitans—? postencephalitic
8	8/23/56	(A. B.)				
9	9/11/56	F. N.	7785R	F	51	Paralysis agitans—? idiopathic; diabetes
10	9/20/56	B. S.	7826R	F	71	Hypertensive vascular disease—recent cerebral vascular accident
11	9/21/56	L. M.	7656R	M	67	Polycythemia vera—old cerebral vascular accident
12	10/2/56	R. B.	7159R	M	48	Essential hypertension
13	10/9/56	G. M.	7835R	M	48	Rheumatoid arthritis
14	11/30/56	T. D.	7853R	M	60	Diabetes—peripheral vascular insufficiency
15	1/25/57	J. B.	8062R	F	47	Paralysis agitans—? postencephalitic
16	2/1/57	(T. D.)				
17	2/15/57	(B. S.)				
18	2/28/57	H. C.	8103R	F	57	Paralysis agitans—? idiopathic
19	3/5/57	(H. C.)				

* () indicates a second entry of a patient previously cited.

of data was facilitated by the reduction of all radioactivity values to standardized units (29, 30). Experimental readings were adjusted for ambient background and then were corrected for radioisotopic decay, variations in tracer dosage and difference in body weight. Thus blood radioactivity is in units of counts per minute per gram of whole blood per microcurie of administered isotope per 70 kilograms of body weight, while external radiation (radioactivity) is in different units of counts per minute per microcurie per 70 kilograms. The empirical ratio relating the two different units remained fixed within the range of experimental reproducibility, so relative changes

in the time courses of blood and body surface data could be compared, as in Figure 1.

Radiation scatter. External radioactivity over the liver usually reached values 10 to 20 times greater than those over the thigh (cf. Figure 1), indicating that radiations scattered from Mn^{56} outside the field of view of this counter were relatively insignificant. Therefore, analyses of liver area data neglected scatter.

RESULTS

Blood and liver area measurements were performed on 18 occasions in 14 individuals (Table I). In one additional instance (Experiment 17) only blood data were obtained.

A. The disappearance of injected Mn^{56} from peripheral venous blood

Compiled blood disappearance data are presented in the scattergram of Figure 2. Beyond 22.5 minutes along the abscissa the regression represented by the data is regarded as diminishing exponentially. A straight line has been fitted to the points in this interval by the method of least squares, using logarithms of the blood radioactivity values.⁵ Data from times less than one

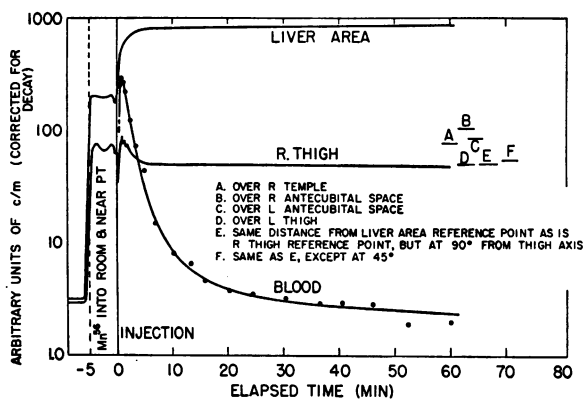
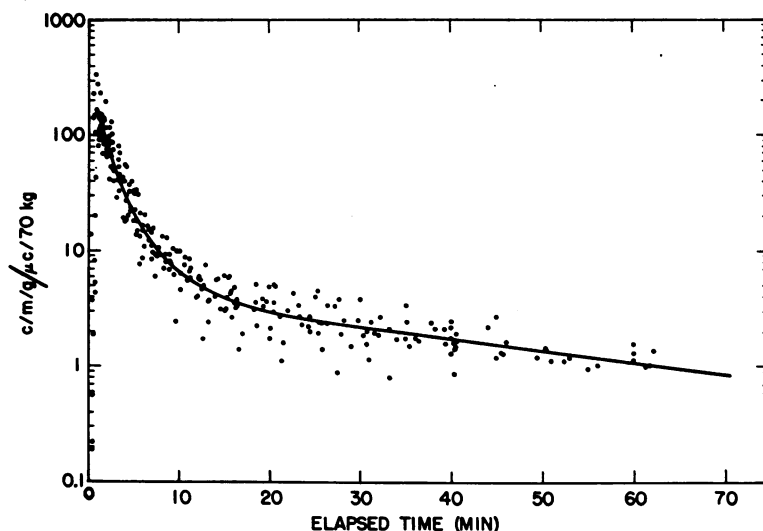


FIG. 1. REPRESENTATIVE EXPERIMENTAL DATA:
PATIENT J. B.

Since the injection site is closer to one counter than the other, note the different readings when Mn^{56} is in position for infusion.

⁵ The resultant least squares regression line of the logarithms (base 10) of the radioactivity against time has a zero-intercept of 0.6375 (log of 4.340), a slope of 0.01007 (log of exp-0.0232), a standard error of estimate

FIG. 2. CLEARANCE OF Mn^{56} FROM THE BLOOD

minute are excluded from the calculated curve, because in individual experiments the peak of radioactivity in the peripheral blood occurs at about one minute or less, with the precise time depending on factors pertinent only to the circulatory mixing process. The remaining blood data between 1 and 22.5 minutes of time have been approximated by a best eye-fit curve, which is drawn to fit smoothly with the least squares regression line at later times. The resultant curve (Figure 2) is termed the "blood clearance curve," whose ordinate values are in standardized blood radioactivity units, as defined earlier. These units have dimensions equivalent to the fraction of administered radioactivity remaining in a gram of blood, after adjustment for variations in body weight.

B. The appearance of Mn^{56} radiation over the body surface

Data from a representative experiment are presented in Figure 1.

1. *Thigh area radioactivity.* The characteristic record of radiation over the thigh reveals an early rise and decay followed by a plateau of activity essentially unchanged for the remainder of the observation period.⁶

(σ) of 0.1377 (i.e., plus 37.1 per cent and minus 27.2 per cent about the mean), and a product moment coefficient of correlation (r) of minus 0.632 ($p < 0.001$).

⁶ The early rise and fall of thigh radioactivity is compatible with the presence of blood-borne Mn^{56} within the

2. *Liver area radioactivity.* The radioactivity in the liver region shows a rapid early rise to a high value which increases only very slightly thereafter. A composite representation of the liver data has been compiled (Table II and Figure 3), using the arithmetic means of the values of individual records at various times after injection.

CALCULATIONS AND DISCUSSION

Analysis of the blood clearance and liver uptake data reported here allows characterization of some important aspects of the metabolic behavior of body manganese.

A. The dynamic metabolic state of manganese

In this portion of the discussion the active turnover of manganese from the systemic circulation will be identified with a rapid transport within the liver and more generally throughout the body.

1. *Rapid movement of manganese from the blood.* The carrier manganese injected along with the Mn^{56} was less than 3 per cent of the normal plasma or blood content (31). Hence it represented an insignificant perturbation of the endogenous physiology. Therefore, a steep decline in

limb. The succeeding plateau appears to reflect radiation scattered largely from the accumulation of tag in the liver and other viscera. This conclusion was based on the virtual lack of change in the "plateau" reading of Figure 1 when the counter was moved in a circle with the liver as center (Curves D, E, and F of Figure 1).

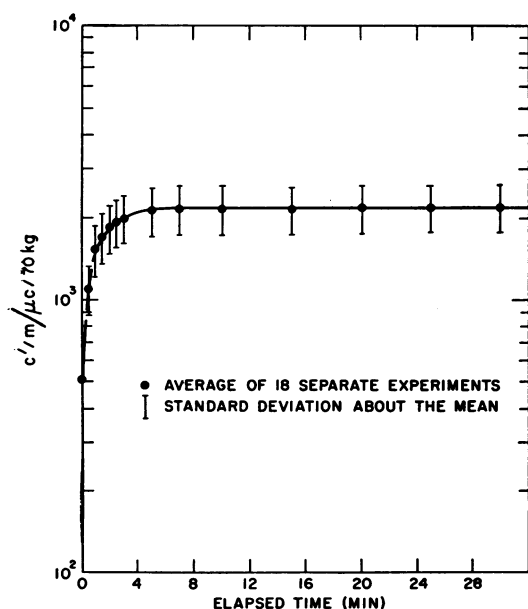


FIG. 3. RADIOACTIVITY OVER THE LIVER AREA AFTER INTRAVENOUS Mn^{56}

Note that the arbitrary counting units of the reference external counter are designated by C' to distinguish them from the readings, C , of the well-counter utilized in the blood work. The length of the bar represents plus-minus one standard deviation about the mean.

blood radioactivity would reflect the rapid movement of both tracer and endogenous circulating manganese to extravascular sites.

Description and significance of the blood clearance curve. Radioactive tracers generally mix with substantially the entire blood volume well within one minute following intravenous injection (29, 30, 32). Figure 2 indicates that much of the radiomanganese initially in the blood is cleared within a minute of virtual mixing, with very little remaining by 45 minutes. However, manganese is excreted much more slowly than this (12, 33–37): Hence the high dilution of initial blood radioactivity implies rapid concentration of the element at certain sites. From partition studies of endogenous and tracer manganese (11, 14), these sites can be presumed to be intracellular.

The blood clearance curve of Figure 2 has been analyzed into three exponentially decaying components (38). It is well represented analytically by the equation

$$a(t) = 265e^{-0.77t} + 44.1e^{-0.26t} + 4.34e^{-0.023t}, \quad (1)$$

where $a(t)$ is radioactivity in standardized units (Figure 4). The first exponential component is comparable to the aperiodic time relation of Shepard, Overman, Wilde and Sangren describing the rapid blood clearance of indicators (30). The last component is identical with the exponential (anti-logarithmic) form of the least squares regression line previously calculated. Although the experimental curve has not been determined for $t < 1$ minute, the "virtual" blood clearance can be extrapolated to zero (injection) time.

Rapidity of transcapillary exchange. The steep decline of blood radiomanganese represented by the first term of Equation 1 implies that in reality the tracer never quite achieves uniform mixing with the entire blood volume, even after several minutes, because along each capillary there must be a strong gradient of radioactivity (29). Furthermore, should this gradient reach zero before the entire length of the capillary be traversed, the transendothelial passage of manganese would actually be more rapid than the calculated value represented by the exponential coefficient, 0.77-min^{-1} . Keeping in mind the distinction between the net movement of small molecules produced by large concentration gradients and other influences and the rates of transfer in both directions through a membrane (39), it is likely that the transcapil-

TABLE II

External radioactivity of the liver (18 experiments)

Elapsed time min.	Mean value $C' \cdot \text{min.} / \mu\text{c.} / 70 \text{ Kg.}$	Standard deviation $C' \cdot \text{min.} / \mu\text{c.} / 70 \text{ Kg.}$
Injection time	507	152
0.5	1,096	223
1.0	1,528	323
1.5	1,698	352
2.0	1,830	362
2.5	1,916	374
3.0	1,978	383
5.0	2,104	418
7.0	2,138	427
10	2,143	427
15	2,132	411
20	2,157	419
25	2,163	415
30	2,171	410
40	2,188	400
50	2,213	373
60	2,216	265

* The units C' refer to counts recorded by the external radiation monitor. These units are to be distinguished from the units, C , which refer to counts recorded by the counter used to determine blood radioactivity.

lary movement is in reality exceedingly rapid, because for water and certain other small molecules the transfer in both directions through the vessel wall occurs at rates that are prodigious in comparison with their rates of *net* transport from the vascular space (39).

The overall turnover rate of blood manganese, therefore, has also been estimated for comparison with that of other small ions which have been studied in detail. R is defined as the fraction of the total intrinsic blood manganese that leaves the vascular compartment in one minute, where

$$\frac{[da(t)]}{(dt)_0} / a_0 = R$$

$$= (-\lambda_1 a_1 - \lambda_2 a_2 - \dots - \lambda_n a_n) / a_0, \quad 2)$$

with a_0 equal to the sum of a_i (29, 32). After substitution from Equation 1, R becomes about 69 per cent.

This value of R calculated for manganese is roughly the same as for other electrolytes, except potassium, as measured in various species (29). Since diffusion or simple filtration, acting alone, would pass all electrolytes at nearly the same rates (29), this similarity is consistent with a diffusion mechanism of transcapillary transport. By contrast, potassium is exchanged much more rapidly than manganese and other ions ($R = 225$ per cent in the rabbit), and for this case more complex transfer mechanisms have had to be invoked (29, 40).

2. Important role of the liver. Prompt hepatic uptake and retention of injected radiomanganese demonstrate that the liver partakes importantly in the swift exchange of circulating manganese with intracellular sites; however, significant extra-hepatic influx of radiomanganese occurs also. This conforms with the organ distribution of Mn^{56} in animals (11, 36, 41).

Intraportal injection studies with tracer manganese in animals demonstrate nearly complete removal of tag in a single transhepatic passage (42). In such a case the fraction of tracer disappearing from blood per minute approaches f/T , where f is the fraction of the entire systemic arterial outflow traversing the liver and T is the time for a single blood volume of tracer to circulate through the heart and appear in the arterial supply or the portal inflow of the liver. Then when other sym-

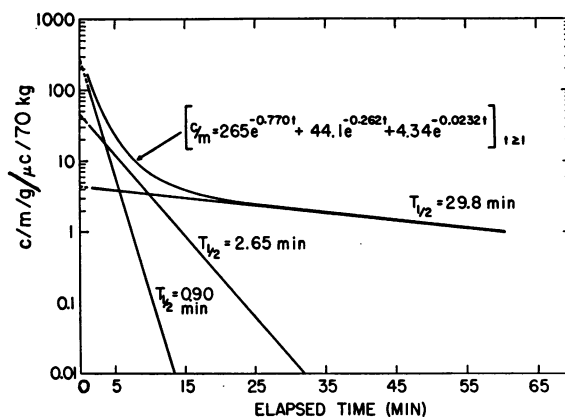


FIG. 4. COMPONENT ANALYSIS OF BLOOD CLEARANCE OF Mn^{56}

bols are employed as before,

$$a(t)/a_0 = e^{-(f/T)t} = e^{-\lambda_1 t}, \quad 3)$$

since f/T is equal to λ (43).

In man the resting cardiac output has been reported variously as about 3 to 6 liters per minute (44, 45), with a total blood volume of 5.0 to 5.5 liters (44, 46). Therefore, one minute represents an apparent average circulation time, T .⁷ λ_1 can be taken from Equation 1, and f then becomes $\lambda_1 T$, or 0.77. However, it is apparent that 77 per cent of the cardiac output cannot traverse the liver, whose total blood flow has been calculated by several methods as about 1,100 to 1,800 ml. per minute (48, 49). It is more reasonable to assume that Mn^{56} is removed from the blood by capillary beds elsewhere equally as fast as it is cleared by the liver.⁸

⁷ The exact determination of T is impossible, since its physical meaning is obscure. Different circulatory pathways may not possess identical circulation times, so several circuits of blood over some shorter routes may occur while a single circuit is being made through some longer pathway (29, 47). For example, the shortest complete circulation time in man is about 21 seconds (range of 12 to 26 seconds) (44); whereas the apparent mean circulation time approximates one minute.

⁸ In fact, assuming the turnover value of manganese to be about 70 per cent (Equation 2) with an hepatic blood flow of 1.5 liters per minute (44, 49) out of a total cardiac output of 4 liters per minute (44, 45), it is found that $0.70(1.5/4.0)$, or about 25 per cent of injected Mn^{56} should accumulate in the liver, provided the intrinsic hepatic clearance is efficient. As a check upon the present calculations, this figure agrees reasonably with the distribution found by Maynard and Cotzias (11, 42) and with that estimated from the data of others (34, 41).

3. *Homogeneity of all pathways of rapid exchange of manganese.* The blood clearance of an intravenously injected radioisotope reflects its movement to *all* organs with which there is significant exchange. An identical time-dependence of radiation recorded over a body surface would imply that these same mechanisms of exchange also produced isotope retention within the tissues whose uptake was thereby being measured. This hypothesis has received general experimental support (48, 50–52).

Blood and liver kinetics of Mn^{56} can be correlated by expressing the liver uptake curve as an enantiogram of the difference between liver activity at a given moment and the final equilibrium ("plateau") value, plotted against time (48) (Figure 5). Then over the first four minutes or so Figure 6 shows the blood and liver kinetics to be virtually identical, and even at later times the dif-

ferences are small. The clearance of the bulk of injected Mn^{56} from the peripheral blood therefore reflects the same means of manganese transport that account for most of its accumulation by the liver.

Extension of the above reasoning implies that most of the influx of manganese into other organs also proceeds in the same fashion as in the liver. Hepatic uptake has been shown to account for the disappearance of well under half of the Mn^{56} from blood. The parallel activity curves can be explained alternatively only by the existence of extrahepatic pathways of rapid manganese interchange which are significantly different in nature from those existing in liver but which possess, nevertheless, virtually identical overall kinetic behavior. This is unlikely.

B. The active role of mitochondria in manganese metabolism

In animals early radiomanganese distribution is characterized by preferential mitochondrial accumulation (11, 42). In the present experiments a single mode of manganese metabolism has been

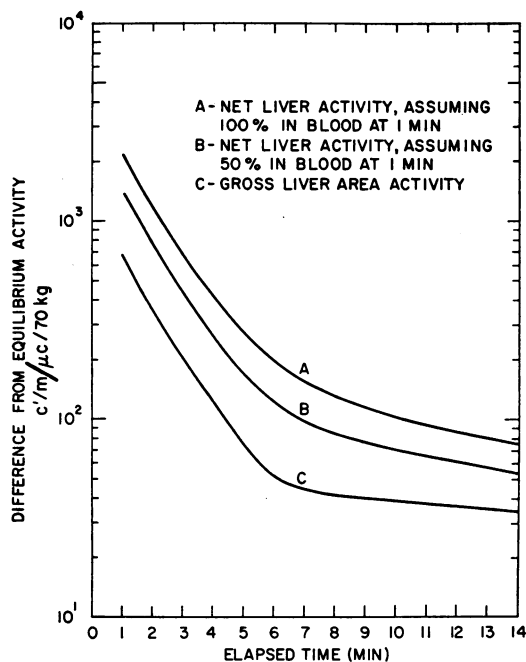


FIG. 5. APPROACH OF LIVER ACTIVITY TOWARD EQUILIBRIUM

The liver area counter also detects Mn^{56} in tissue adjacent to the liver. At the time of maximum blood radioactivity (*ca.* one min.) Curve A assumes all detected activity to derive from tracer still circulating, while B represents a less severe assumption. A and B are calculated from Figure 3, taking circulating Mn^{56} to decline as in Figure 2. Intrinsic hepatic radiomanganese uptake presumably lies within the range A-C.

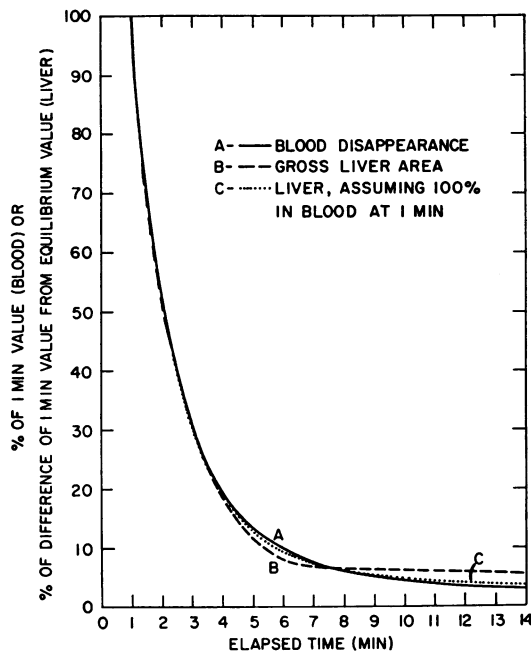


FIG. 6. COMPARISON OF EARLY BLOOD AND LIVER KINETICS

The kinetics of Mn^{56} movement in blood and liver are nearly congruent regardless of whether liver area (B) or calculated organ uptake data (C) are employed.

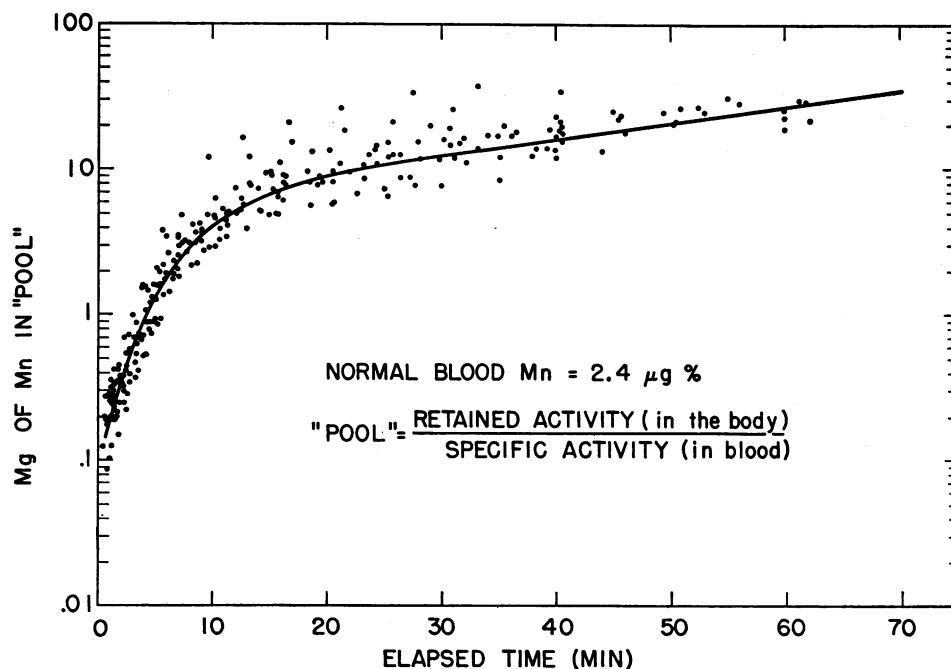


FIG. 7. "APPARENT" EXCHANGEABLE MN "POOL"

shown to determine overall tracer distribution in the body shortly after injection. It therefore appears that in man the mitochondria of all organs participate importantly in the rapid interchange of endogenous tissue manganese with that circulating in the blood.

It has been shown earlier that the first of the analyzed exponential components of the Mn^{56} blood disappearance curve probably represents transcapillary exchange, and later it was inferred that mitochondrial assimilation dominates the transfer of tracer during the first hour. Since the first term of the blood curve has been otherwise identified, whereas the third term does not suggest an exchange as rapid as the rate of mitochondrial uptake, it is probably the second component of the clearance curve (*cf.* Figure 4 and Equation 1) that reflects strongly the movement of injected radiomanganese into the mitochondria themselves. However, that component's slope and intercept cannot as yet be interpreted in a quantitative fashion.

Insofar as rapid manganese exchange in tissue signifies mitochondrial function, some measure of this intracellular parameter may be determined in man, using the methods reported here. The

total mass or activity of mitochondria⁹ may be indicated by the initial portion of the radiomanganese blood clearance curve, and particularly by the second exponential component analyzed from it; and the liver area uptake may provide a similar measure of hepatic mitochondria. Independent substantiation would be highly desirable, but present cellular fractionation techniques (11) lack sufficient time resolution to confirm this relationship directly in animals.

C. The labile physico-chemical status of body manganese

Each of the recognized essential mineral micronutrients other than manganese¹⁰ has been demonstrated as a strongly bound intrinsic component of one or many naturally occurring metalorganic compounds (2, 16, 18, 19, 53). In contrast, no manganese metalorganic component has yet been definitely identified in animal tissues (2, 16, 18, 19, 53).¹¹

⁹ Exclusive of brain, because of the intervening blood-brain barrier.

¹⁰ Iron, cobalt, copper, zinc, and molybdenum.

¹¹ Since this paper was submitted for publication, evidence has been adduced that strongly suggests the presence

The mass of manganese apparently interchanging with Mn^{56} can be represented by a dilution "pool" (54) (Figure 7). Manganese excretion is negligible over the first hour (12, 33-37). Retained activity (Figure 7) is therefore taken as the entire radioisotope dose.

Data on manganese in blood are meager (3); but recently Bowen has perfected a technique of improved resolution and sensitivity wherein small blood samples are radioactivated by reactor neutrons, followed by conventional carrier chemical separation processes and then by counting the isolated Mn^{56} (31). The concentration of manganese has been determined thereby to be $2.4 \pm 0.8 \mu\text{g. per 100 ml.}$, divided roughly equally between cells and serum. This represents the lowest reported value (3, 4, 55, 56).

In the absence of individual values, that of 0.024 $\mu\text{g. per gram}$ from Bowen (31) has been assumed. Thence from the individual data of Figure 2 a dilution "pool" was calculated (Figure 7).

Recent reports cite a total body manganese content of 11 to 20 mg. (2, 46). Extrapolation from human (7) and animal (13) tissue measurements places the body manganese at 9.5 to 12.0 mg. and 22.5 to 27.2 mg., respectively. In any case, by 45 to 60 minutes the Mn^{56} interchanges with a pool representing roughly all the endogenous body manganese (Figure 7).¹²

The rapid dilution of tracer (Figure 7) indicates the absence of a significant nonexchangeable portion of the total body manganese, and conversely, that most body manganese must reside in relatively labile metal-protein complexes or similar configurations compatible with its known chemistry (13-17).

SUMMARY AND CONCLUSIONS

Combined studies of blood clearance and radiation measured over the liver in humans following the intravenous injection of tracer doses of Mn^{56} showed a consistent pattern. The transfer of manganese from blood into intracellular sites was rapid throughout the body.

of a small amount of stable manganese-porphyrin complex in man (Borg, D. C., and Cotzias, G. C. Submitted for publication).

¹² Utilization of the lowest value cited for blood manganese yields a minimum estimate of exchangeable pool size.

The data showed further that most or all of the endogenous manganese became available for interchange with tracer within an hour. Hence most of the element within the body must exist in dissociable chelates or other relatively labile intracellular combinations. Considering the constancy of tissue concentrations of manganese, this demonstrates that manganese metabolism is under homeostatic control.

At least three, and probably four, apparent body compartments participate to some extent in the distribution of Mn^{56} within the first hour. The first component analyzed from the blood disappearance curve probably reflects the net transcapillary passage of the element.

Tracer concentration falls steeply in the capillaries, and about 70 per cent of the normal blood manganese leaves the circulation each minute. The transendothelial transport of manganese appears to be by passive diffusion. It follows that net turnover from the blood is perfusion-limited, despite subsequent intracellular concentration.

Identical mechanisms appear responsible for most of the swift inflow of manganese into liver and simultaneously into other organs. Mitochondrial uptake throughout the body is regarded as underlying the bulk of this rapid tissue retention, which largely determines a second component analyzed from the blood data as well.

A mitochondrial role is in accord with the known biochemistry of manganese *in vitro*. Therefore, the procedures reported here are proposed as a method for measuring mitochondrial mass or function in patients.

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