Slow Turnover of Manganese in Active Rheumatoid Arthritis Accelerated by Prednisone

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ABSTRACT Total body and area counts of intravenously injected ^{54}Mn were measured periodically in 29 in-patients. A heterogeneous group of 19 control patients showed fair reproducibility in the immediate distribution, and considerable individual variance in the subsequent loss of the isotope. Eight studies of the effects of feeding excesses of manganous sulfate to five patients showed acceleration of the rate of loss of the radioisotope from the whole body and the liver. These findings seem compatible with the presence of control mechanisms in man, operating to vary the metal's excretion, while tending to preserve constancy of its concentration in tissues.

Slow turnover rates of the metal were demonstrated in seven out of eight patients with active rheumatoid arthritis, in one with hydralazine disease, but not in one arthritic undergoing an impressive, spontaneous remission. Statistically significant differences were encountered in the measurements of ^{54}Mn turnover of the total body, the thyroid, and the liver.

Administration of prednisone induced clinical improvement and significant acceleration of these turnovers. Slow turnovers are characteristic of nutritional manganese deficiency. Therefore, serum and blood manganese determinations were performed by neutron activation analysis on 14 control patients, and on six patients with active rheumatoid arthritis. A statistically significant *elevation* of the red cell manganese concentration was encountered in patients with rheumatoid arthritis. This argued against the presence of classical trace-metal deficiency and called for an alternative explanation of these findings.

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INTRODUCTION

Enzymes critical to mucoprotein synthesis are activated by manganese (1–3), and, conversely, defective mucopolysaccharide synthesis is found in manganese deficient animals (4, 5). These mechanisms underlie the gross mesenchymal aberrations which characterize manganese deficiency (6).

The postulate that abnormal manganese metabolism might be present in human collagen disease rests on the following: (*a*) Rheumatoid arthritis displays abnormalities of mucoprotein and mucopolysaccharide metabolism (7–10); (*b*) Another collagen disease, the hydralazine syndrome, appeared linked to manganese deficiency on the basis of animal studies (11, 12); (*c*) Induction of the hydralazine syndrome seems to depend upon the genetic substratum (13); and (*d*) Genetic factors may interact with environmental ones in the development of manganese deficiency, as was proven by the manganese-dependent defects of the pallid strain of mice (14, 15).

Studies of manganese metabolism in animals have shown that the liver plays a cardinal role in the distribution and excretion of this metal (16, 17). Furthermore, both glucocorticoid hormones (18) and adrenocorticotropin ACTH (19) shifted manganese from the liver to the carcass in mice. It seemed worthwhile to seek similar effects in man. Among diseases which respond to hormonal treatment, rheumatoid arthritis was chosen following the discovery of slow turnover of ^{54}Mn

in a patient who developed arthritis while receiving hydralazine.

The present paper suggests the existence of biological controls for manganese in man, similar to those in other mammals (16–20). Thereafter it documents a slow turnover of this metal in one patient with hydralazine disease and in seven with active rheumatoid arthritis. This metabolic defect proved corrigible by treatment with prednisone.

METHODS

Patients. 11 in-patients provided samples of blood and plasma for supplementary determinations of manganese, however they did not participate otherwise in this study. Among the 29 patients who took part in at least one isotopic run (Table I), 16 served as controls for the arthritics. Several diagnostic categories were deliberately represented, including two patients with Wilson's disease. Healthy, working individuals were deemed inappropriate as controls.

All patients admitted remained in a metabolic ward during these studies. Caloric intake was satisfactory as a rule. Manganese intake was intentionally not fixed, since the biological control of this metal was under scrutiny. Exceptions were patients 4 and 9 (Table I). Patient 4 had received steroid therapy before admission and was consuming a low caloric intake after its interruption. She was placed, therefore, on a fixed 2600 calorie intake (high Mn intake). Patient 9 is discussed in detail below.

TABLE I

Patients, Diagnoses (Dx), Measurements, and Drugs

Case No.	Dx	Measurements 1	2	3	4	5	6	Observations with $^{54}MnCl_2$	$^{55}MnSO_4$	Prednisone (initial dose)
									mg/day	*mg/day*
1	A	+	+	+	+	+	+	1		50
2	A	+	−	+	+	+	+	1		20
3	A	+	+	+	+	−	+	1		50
4	A	+	−	+	+	−	+	1		50
5	A	+	−	+	+	+	+	1		50
6	A	+	−	+	+	+	+	1		100
7	A	+	−	+	+	+	+	1		100
8	A	+	−	+	−	−	−	1		40
9	A	+	−	+	−	−	−	1		40
10	B	+	−	+	+	+	+	2	2000	
11	B	+	+	+	−	+	+	2		
12	B	+	−	+	+	−	+	1		
13	B	+	−	+	+	+	+	1		
14	B	+	+	+	+	+	+	1		
15	BG	+	+	+	−	+	+	3	1200	
16	B	−	+	+	−	+	+	1		
17	B	+	−	+	−	−	−	1		
18	B	−	+	+	−	+	+	1		
19	B	+	+	+	+	+	+	1		
20	B	−	+	+	−	+	+	1		
21	B	−	−	+	+	+	+	1		
22	B	+	+	+	+	+	+	1		
23	C	+	+	+	−	+	+	2	1200	
24	D	−	+	+	−	−	+	1		
25	E	−	+	+	−	−	+	1	1600	
26	E	−	+	+	−	+	+	1		
27	F	−	+	+	−	−	+	2	2000	
28	B	+	−	+	−	−	−	1		
29	B	+	−	+	−	−	−	1		

1, Total body counts (steel room); 2, Total body counts (crystal at 155 cm); 3, Counts over liver; 4, Counts over thyroid; 5, Counts over thigh; 6, Counts over right ear. A, Rheumatoid arthritis; B, Parkinsonism; C, Friedreich's ataxia; D, Diabetes mellitus; E, Wilson's disease; F, Cerebral vascular thrombosis; G, Hydralazine syndrome. Repetition of the ^{54}Mn observations took place only after the radioactivity had reached negligible levels. Patient 25 received the $^{55}MnSO_4$ about 1 yr after the injection of ^{54}Mn. Separate series of measurements were performed when $^{55}MnSO_4$ was to be readministered to patients 23 and 27.

In addition to semi-daily visits, a battery of tests [1] was performed periodically to follow up the clinical state and to detect possible drug toxicity.

The supportive drugs given included laxatives, antiparkinsonian agents, hypnotics, and salicylates [2] as occasional analgesics only. The laxatives produced minor but sharp drops in the counting rates of the whole body, the liver, or of both which cancel out in the scattergrams presented here.

The effects of $MnSO_4$ administration on the turnover of injected ^{54}Mn were assessed on five control individuals after completion of at least one isotopic run. Administration of the doses shown in Table I was continuous over the time periods shown in Fig. 2.

The effects of prednisone on the elimination of ^{54}Mn were induced only after a significant base line was established, namely about 1 month. Each patient served as his own control. The initial oral doses of prednisone (Table I) were gradually diminished in the manner shown on Fig. 5. All patients in whom steroid therapy could not be postponed for a month were excluded from this report. This restraint has excluded all patients with lupus erythematosus disseminatus. The patients understood the consequences of these procedures and gave their informed consent.

Isotopes. The long-lived ($t_{\frac{1}{2}} = 314$ days) gamma ray–emitting, carrier-free $^{54}Mn^{++}$, as well as the nonradioactive, natural $^{55}Mn^{++}$ ($MnSO_4$), have been discussed earlier (16–20). Each batch of radioisotope was first tested for toxicity in rabbits or mice. Calibrated doses ranging from 24 to 50 μc were dissolved in 1 ml of 0.9% NaCl solution. These were injected into the distal tubing of an intravenously running saline solution. At times some of the isotope was injected subcutaneously. These runs were also excluded from the present report.

The calculated total body radiation dose received at infinite time by these patients was 0.9 rad/50 μc. The calculated dose to the liver and to the immediately adjacent structures was 9.6 rad at infinite time.

Total body counting. Total body counting was performed shortly after injection of the isotope and periodically thereafter. In many instances a NaI (TI) crystal (1 3/4″ × 1 3/4″) located at a distance of 155 cm from the umbilicus and attached through appropriate circuitry to a scaler was used. Such total body measurements are referred to as "crystal at 155 cm." However, the sensitivity of this detector was not uniform over the whole body at the level of the bed (21). The counting efficiency of the ^{54}Mn standard, 40 cm from the umbilical position, was 80% of the response at the umbilical position; hence, a whole body counting assembly located in a steel room was used thereafter (23). This assembly had much more uniform counting geometry (23). Interconversion of the two sets of data proved impossible; thus they are analyzed and presented separately.

Area counting. The crystal used for total body counting was placed directly on marks reflecting maximal radioactivity over the liver, the thyroid, above the right ear, and over the right midthigh (22). The liver and thyroid were selected to represent organs with vigorous mitochondrial metabolism, whereas the ear and the thigh represented primarily mesenchymal structures (21, 22). The Picker Magnascanner with a 2″ × 2″ NaI (TI) crystal, without the focusing collimator, was used on patients 6, 7, 8, and 9 (Table I) for area counting.

Manganese determinations. Manganese determinations were performed on whole blood and serum samples by means of neutron activation analysis. The method used was developed previously (24) and the precautions against contamination and loss were specified elsewhere (25). The advantages of this cumbersome method are: (*a*) high sensitivity, and (*b*) prevention of contamination of manganese-poor samples with exogenous metal. Red cell manganese concentrations were computed from the corresponding whole blood and hematocrit values without correction for trapped serum. Analysis of variance was performed with a Fortran IV Program (Code name, HIER) using a CDC 6600 computer.

Processing of isotopic data. The counts obtained in the steel room and those obtained over the liver and thyroid could be determined initially with a precision of 1%; those with the crystal at 155 cm, 3%; those over the ear and thigh, 2 and 1%, respectively. Corrections were applied for background, decay, and occasional drifting of the instrumental response.

The individual data were first plotted as semilogarithmic plots of cpm/μc of ^{54}Mn injected against time (Table II), without corrections for the patients' body mass (22). Such a plot is shown on Fig. 5.

The plots shown in the rest of the figures were normalized as 100% at 0 time. Note that zero time is defined differently in different experiments.

Two sets of calculations were made on these data: (*1*) The data, expressed as cpm/μc, were fitted to single straight lines by means of least squares. For this a Fortran II program (code name, GLLS) was used. Table III shows the mean biological half-lives; the coefficient of correlation with the straight line; and the P values comparing the two groups. The coefficient of correlation was not quite satisfactory, because the data of several individuals (mostly the controls) delineated complex

[1] Hemoglobin, hematocrit, RBC, WBC, differential, reticulocyte, and platelet count; Coomb's test, and sedimentation rate. Urine analysis, gram stain of sediment, and urine culture; stool guiac. Blood urea nitrogen, urea and creatinine clearances. Serum electrolytes, uric acid, and protein-bound iodine. Total cholesterol and esters, bromsulfalein, cephaline flocculation, thymol turbidity, prothrombin, serum bilirubin, total protein and A/G, C-reactive protein, antistreptolysin, rheumatoid factor, LE test, ECG, radiologic examinations, serum electrophoresis, sight screening and audiograms.

[2] Control patient 11 (Table I) received 8.0 g/day of aspirin for 3 months during one of the studies, while total body and area counting had been in progress. The rate of loss of ^{54}Mn from the liver became significantly *accelerated.* This was in accord with observations on patient 9 (see text) and operated to *diminish* the differences between arthritics and controls.

TABLE II

Uptake of ^{54}Mn by the Whole Body and the Regions Indicated

	Control			Rheumatoid			
	n		Mean	n		Mean	P
1 Steel room	9	21923	±930	9	19727	±832	0.10
2 Crystal at 155 cm	10	30.6	± 0.6	2	30.5	± 1.5	0.95
3 Liver	16	913.5	± 45	9	907.5	± 58	0.94
4 Thyroid	6	216.6	± 24	7	204.4	± 13.8	0.65
5 Ear	10	118.4	± 3.59	5	104	± 7.3	0.07
6 Thigh	13	56	± 2.4	7	54.8	± 4.9	0.80

These values were computed as cpm/μc of ^{54}Mn. n, number of patients.

exponential curves. Therefore, an additional intercomparison was made between the patients with rheumatoid arthritis vs. the controls. This was performed as follows.

(*2*) The time of the injection of the isotope was taken as the reference point. Subsequent points were expressed as per cent of that value (see Fig. 1). Lines were fitted by French curves to each set of data from each patient. Points collected on these lines represented the readings on the controls and the arthritics at 5 day intervals. These readings were submitted to statistical analysis by Student's *t* test. *P* values were determined with the XEQ program (Table IV) generously provided by Dr. Keith Thompson of this Laboratory.

The changes in counting rate brought about by the administration of manganous sulfate (Fig. 2) or prednisone (Figs. 4 and 5) were assessed as follows: the radioactivity determined at the time of the administration of these agents was the point of reference. The measurements which preceded and which followed were expressed as per cent of this point.

RESULTS

Turnover of ^{54}manganese. 16 of the runs on 16 patients who did not receive either manganous sulfate or prednisone are included in the control group. As shown on Table II, the initial distribution of the injected radioisotope was fairly reproducible from one patient to the next. However, the rates of elimination showed considerable individual variance. This is shown in Fig. 1 (1 A, 2 A, 3 A) in which the first count was the point of reference for all subsequent measurements. Table III lists the biological half-lives of the sum of our data (after fitting them by the method of least squares) as well as the *P* values comparing the two groups. The steel room whole body counter recorded a slower rate of ^{54}Mn excretion than did the crystal at 155 cm (Table III). This was due to the better geometry achieved in the steel room by comparison with the single small crystal, which reflected the liver turnover more heavily.

In each individual record, the liver showed the most rapid turnover (16–20) with the thyroid ranking as second. By contrast, the ear and the thigh showed much slower rates of loss of ^{54}Mn (21, 22). These were almost identical to each other, despite the higher initial radioactivity detected over the ear (Table II). Hence, the intrinsic

TABLE III

Mean Biological Half-Lives in Days

	Control		Rheumatoid arthritis		
	$t_{\frac{1}{2}}$	*r*	$t_{\frac{1}{2}}$	*r*	*P*
1 Steel room	59	0.58	118	0.52	$0.01 < P < 0.05$
2 Crystal at 155 cm	39.5	0.91	66	0.67	NS*
3 Liver	37.8	0.67	121	0.54	0.0002 (0.0029)
4 Thyroid	48.6	0.85	67.8	0.42	$0.005 < P < 0.01$
5 Ear	172.3	0.49		0.06	NS
6 Thigh	161.1	0.32	120	0.35	NS

$t_{\frac{1}{2}}$, mean biological half-lives in days; *r*, coefficient of correlation of data to straight lines on semilog plots obtained by least squares. NS, not significant (*P* 0.05). The *P* values were calculated from the sum if the points on each curve representing the control and the arthritic groups (method 1). The parenthesis included patient 4.

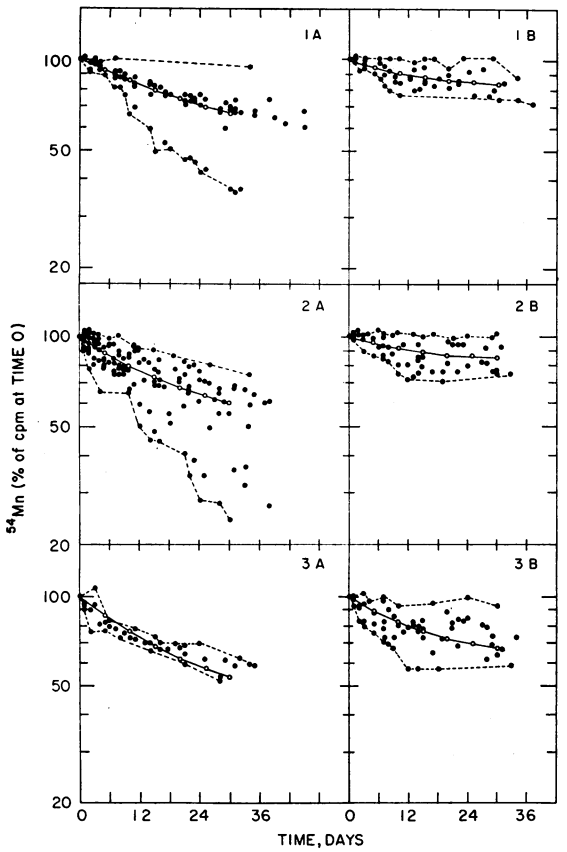

FIGURE 1 Per cent of ^{54}Mn remaining with passing time. A, Controls; B, Rheumatoid arthritis; 1, Steel room; 2, liver; 3, thyroid. ●—● = envelope; ○—○ = average.

manganese turnover of brain was masked again by that of the mesenchymal and ectodermal structures, as had been the case earlier (21, 22).

The question dealt with next was whether administration of stable, natural ^{55}Mn would accelerate the loss of the radioisotope ^{54}Mn, as it had in experimental animals (16, 20, 26). Fig. 2 shows that manganous sulfate loads induced initially a sharp decline of whole body and liver radioactivity. This was followed by a slower rate of loss, in spite of continuous administration of the manganous salt (20). Furthermore, the relative amount of tracer lost was decreased by increasing the time interval between injection of ^{54}Mn and ingestion of ^{55}Mn (20). Scrutiny of each individual record showed that no acceleration was induced in the loss of the tracer from the ear or the thigh.

Slow turnover in rheumatoid arthritis. The first indication of disturbed metabolism of manganese was elicited on a patient who, while under study, developed arthritic manifestations from hydralazine. Records obtained before and during hydralazine administration are shown in Fig. 3. Her control record is included among the other controls (Table I, patient 15). While on a maximum of 800 mg of hydralazine, she developed limitation of motion, pain, swelling, and redness of small joints, accompanied by fever and dyspnea. Laboratory changes included positive LE and Coomb's tests, increased sedimentation rate, and albuminuria. These manifestations disappeared gradually, but in toto, upon stopping the drug. They have not reemerged during 7 yr of follow-up.

Among the arthritic patients, the distribution of the isotope was again fairly reproducible (21) and quite similar to that of the controls (Table II). Differences between these groups became evident upon analysis of the rate of loss of the isotope as a function of time. Only one control patient showed a total body loss similar to the arthritics. Among the seven arthritics under present consideration, the curves obtained on patient 4 were the only ones that approximated those of the control group (see Methods).

In the other six patients, each curve that represented the whole body, the liver, or the thyroid showed relatively prolonged mean biological half-lives, as illustrated in Fig. 1 (1B, 2B, 3B) and Tables III and IV. The records obtained over the ear and the thigh were similar to those of the controls (21, 30). Statistical evaluation according to method 1 (Table III) showed that measurements over these primarily mesenchymal structures were not significantly different between controls and arthritics. By contrast, (Tables III and IV) the data obtained in the steel room showed that the total body turnover of manganese in the arthritics was significantly slower than in the controls. Similarly, the rate of loss measured over the thyroid appeared to be slower in these patients. This trend was strengthened by the ^{54}Mn counts obtained over the liver: even when all seven patients were included, the radioactivity remaining in the livers of the arthritics after 1 month was higher than that of the controls (P, 0.003).

The patient with hydralazine syndrome had a slower turnover only during the active phase of the disease. This raised questions with regard to active vs. inactive rheumatoid arthritis. Therefore, an arthritic who had recently undergone a sudden,

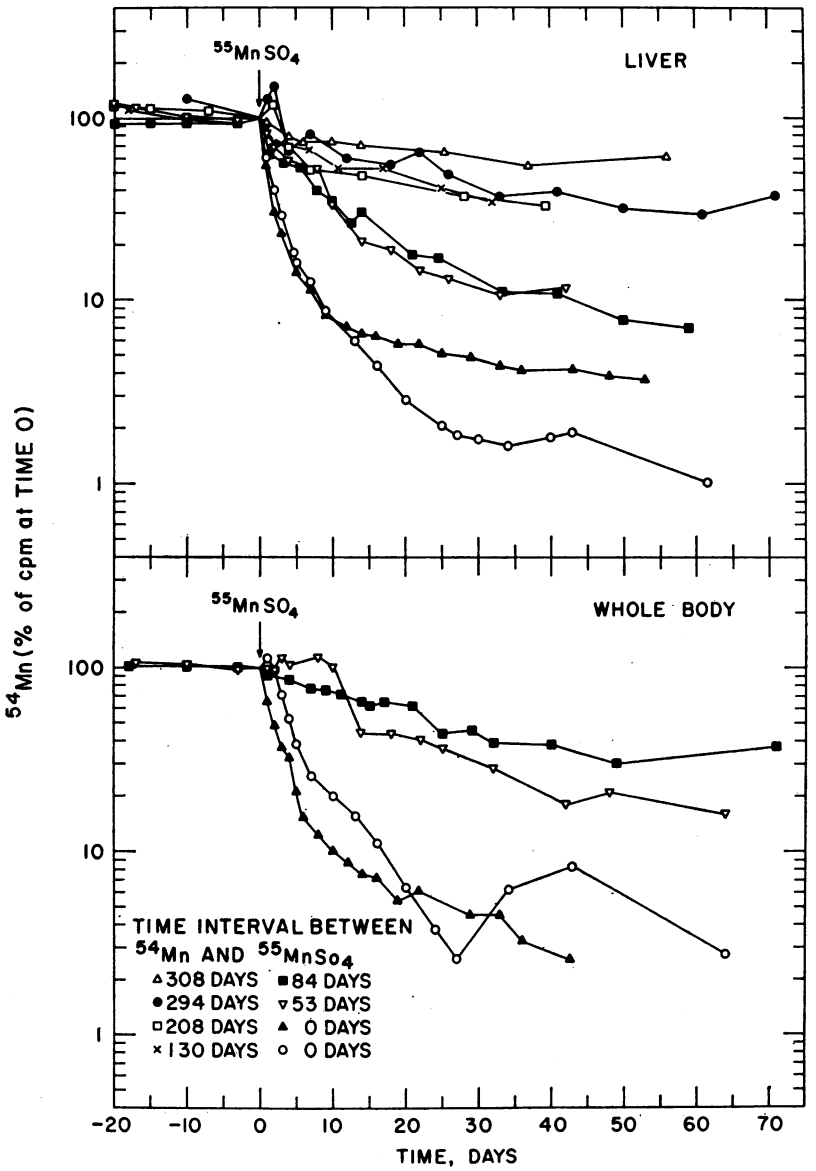

FIGURE 2 The effect of $MnSO_4$ on the area counts over the liver (upper panel) and those obtained by the crystal at 155 cm (lower panel). The data were normalized at the point of administering $MnSO_4$.

almost total, spontaneous remission was studied as an additional in-patient (No. 8). She was physically very active and essentially asymptomatic without treatment. She displayed negative tests for C-reactive protein and latex fixation, negative Coomb's tests, antistreptolysin titers of 125, and a corrected sedimentation rate of 22 mm/hr. Yet, she showed radiological evidence of previous rheumatoid arthritis. The half-lives of the total body and the liver were markedly shorter than the entire range of control patients (Table III), namely, 40 and 20 days, respectively (compare with the effect of prednisone).

Since salicylates had been given intermittently to most of the seven arthritics, the turnover of manganese was investigated in an additional patient (No. 9) with severe, active rheumatoid arthritis, who was willing to be observed without therapy. During a month of observation she received intermittently a maximum of only 100 mg of indocin

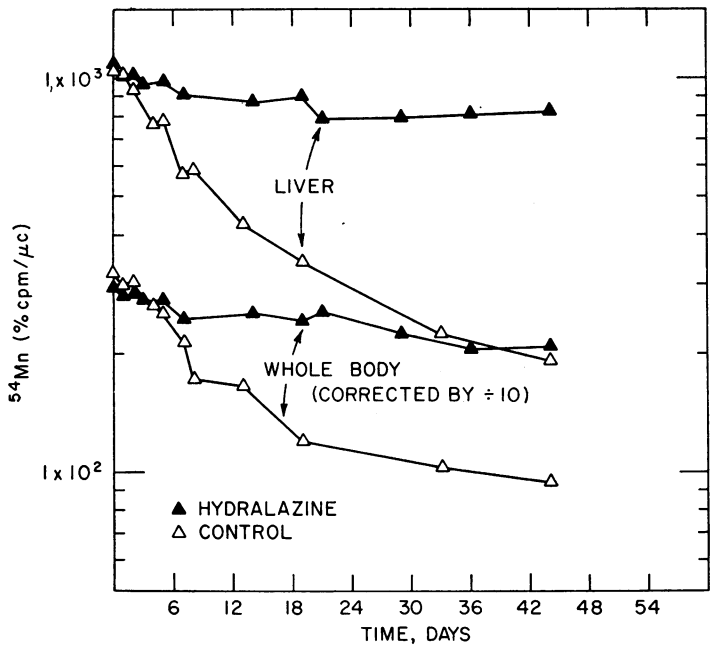

FIGURE 3 Counts of ^{54}Mn over the liver and by the crystal at 155 cm, before (△) and during (▲) hydralazine administration. Different runs on the same patient. (See text.)

per day. Her total body and liver measurements showed ^{54}Mn half-lives of 110 and 310 days, respectively. Upon being given tolerance doses of aspirin (3.0 g/day) over 2 months, the rate of loss from the total body remained essentially unaffected, whereas the half-life over the liver became 101 days. Caloric intake was quite constant during this period, and was kept unchanged during subsequent treatment with prednisone.

Administration of prednisone. The administration of prednisone to the arthritic patients induced an acceleration of both the total turnover and the rate of loss of ^{54}Mn from the liver (Fig. 4) and the thyroid. The data on one of these patients (No. 3, Table I) are shown in Fig. 5 as an example of an individual record. A slow turnover of the metal is evident only in the total body, liver, and thyroid measurements, but not in those obtained over the thigh (compare with Table III). The hormone had accelerated only these slow

TABLE IV

Comparison of the Per Cent of ^{54}Mn Remaining at the Areas and Times Indicated (Method 2)

		Steel room			Thyroid			Liver		
		Mean		P	Mean		P	Mean		P
5 days	Control	92.1	±1.32	0.34	86.2	±0.66	0.25	88.3	±1.19	0.006
	Rheumatoid	94.2	±1.72		89.5	±2.57		95.4	±2.14	
10 days	Control	85.1	±2.44	0.18	76.2	±0.99	0.24	79.7	±1.90	0.004
	Rheumatoid	90.2	±2.63		81.7	±4.28		91.8	±3.40	
15 days	Control	79.0	±3.46	0.09	68.0	±1.10	0.18	73.3	±2.39	0.003
	Rheumatoid	87.8	±3.02		76.0	±5.44		88.6	±4.08	
20 days	Control	73.9	±4.28	0.06	61.8	±1.16	0.13	67.8	±2.78	0.002
	Rheumatoid	85.7	±3.27		71.9	±6.07		86.7	±4.28	
25 days	Control	69.8	±5.05	0.05	57.0	±1.33	0.09	63.4	±3.09	0.002
	Rheumatoid	84.2	±3.54		69.0	±6.44		86.3	±5.40	
30 days	Control	66.6	±5.62	0.05	53.5	±1.44	0.07	59.8	±3.29	0.001
	Rheumatoid	82.8	±3.76		67.1	±6.53		85.3	±5.55	

rates, whereas the rate of loss of the isotope from the thigh may even have become decelerated (18, 19). The latter finding was not clearly evident in all records. The supplementary patient (No. 9) whose caloric intake had been kept constant before and during administration of prednisone (initial dose, 40 mg/day) showed dramatic acceleration of the rate of loss of ^{54}Mn, both from the total body and from the liver. The respective half-lives of 110 and 101 days became 15 and 20 days.

Analyses of whole blood and serum. Previous experiences with animals (16, 17, 19, 20, 26) and man (21, 27) raised questions as to whether the slow manganese turnover rates of the arthritic patients might signal the existence of manganese deficiency in this disease (11, 12). This question emerged only after the isotopic records had been fully analyzed and when some of the patients were discharged. Therefore, the relatively few analyses of blood and serum manganese obtained were supplemented with additional ones.

These data are shown in Table V. Surprisingly, the red cell manganese of the arthritics was significantly *higher* than it was in the controls ($0.01 > P > 0.05$).

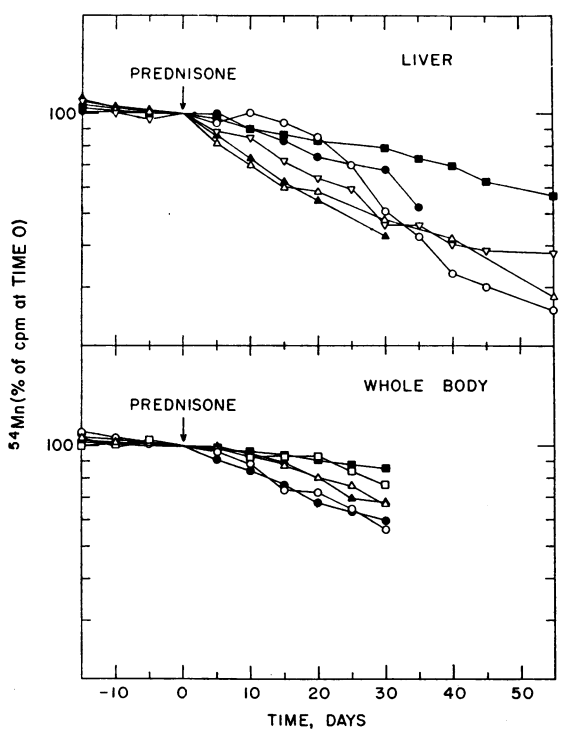

FIGURE 4 Semilogarithmic plots of the effect of prednisone on area counts over the liver, and whole body counts obtained in the steel room on arthritic patients, normalized at the time of prednisone administration.

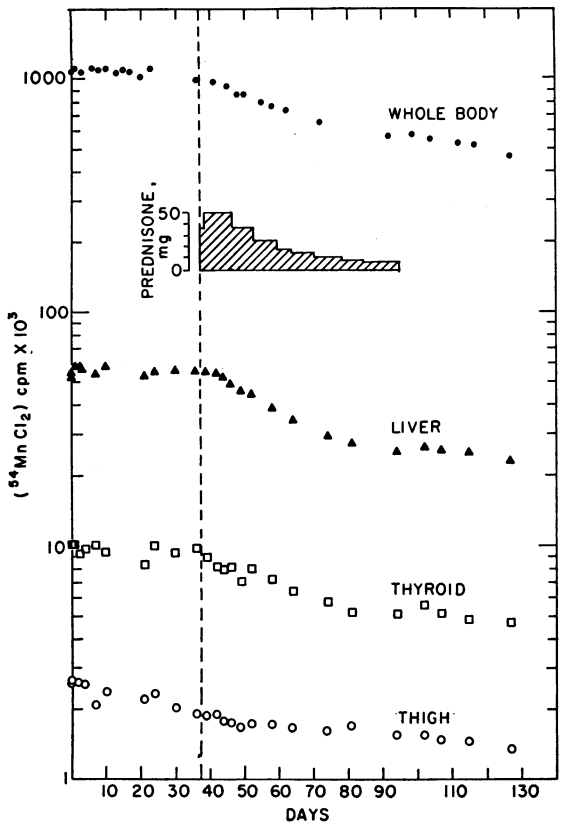

FIGURE 5 Semilogarithmic plot of data obtained on patient 3 (Table I). Note that, whereas the slow rate of loss of the isotope from the whole body, the liver, and the thyroid became accelerated by the hormone, the more rapid loss from the thigh became decelerated.

DISCUSSION

The control group was heterogeneous and even included two patients with Wilson's disease. Their

TABLE V
Blood Manganese

	Control			Rheumatoid		
	n_1	n_2	Mean	n_1	n_2	Mean
Whole blood	14	63	9.84 ± 0.4	6	16	14.99 ± 2.4
Red cell	14	46	23.57 ± 1.2	6	14	33.60 ± 5.0
Serum	14	55	1.42 ± 0.2	6	16	1.69 ± 0.4

Concentrations of manganese in μg/liter of whole blood, red cells, and plasma. n_1, number of patients. n_2, number of analyses.

manganese intakes were deliberately not fixed. Still, the initial distribution of the injected tracer was fairly reproducible between one patient and the next. This was in accord with findings on working manganese miners, on ex-miners with chronic manganese poisoning, and on healthy Chilean hospital workers (21, 27). A fair reproducibility was displayed also by the blood and serum manganese levels, which were commensurate with those of normal individuals (21, 24, 25).

By contrast, the rates of loss of the tracer from various regions were variable and some could be accelerated by feeding manganous sulfate. These findings concord with the following laboratory and clinical experiences.

In experimental animals, acceleration of the tracer's distribution and excretion was readily induced by manganous salts (16–20, 26). In each instance, isotopic exchange was prominently displayed by the parenchymatous organs, whereas the carcass appeared refractory to it. The degree by which elimination of the tracer was accelerated depended upon: (*a*) the magnitude of the manganese load; (*b*) the time that elapsed between injection of ^{54}Mn and ingestion of ^{55}Mn. As in the present experiments on humans, progressively smaller proportions of the tracer remained exchangeable in animals with passing time. In the mouse (19), acceleration of the excretion tended to maintain constant manganese concentrations in tissues, albeit with limited success when the manganese loads were massive.

The turnover data on the present controls agree with those on Chilean hospital workers and on ex-miners suffering from chronic manganese poisoning (21, 27). Furthermore, these data contrast both with those on the arthritics (see below) as well as with those on healthy miners exposed to manganese dust (21, 27). The latter showed fast elimination of the tracer from the whole body and the liver. Elimination became decelerated upon removing the miners from the mine (21, 27). Again, the differences among these groups were displayed by the measurements of the whole body and the liver. The sum of the above concords with the conclusion of Schroeder, Balassa, and Tipton that specific biological controls exist for manganese in man (28).

The patient with hydrolazine syndrome and seven of the eight patients with active rheumatoid arthritis showed slower rates of loss of ^{54}Mn than did the controls. The whole body and the parenchymatous organs did display this difference, whereas the thigh and the ear did not. It should be noted that several weeks had to elapse before losses of ^{54}Mn from the regions studied could be defined. By sharp contrast, within 10 min after intravenous injection more than 90% of the ^{54}Mn was cleared both from the circulation of animals (17, 29) and of man (21, 22). The radioactivity became preferentially localized in the mitochondria (18, 29). Therefore, the slow turnover of the arthritics may not be explained on the basis of slow blood clearance. On the contrary, it must be assigned to a delay at the intracellular level. The parenchymatous tissues have vigorous mitochondrial metabolism, whereas the mesenchymal tissues do not. It is serviceable to postulate that this delay occurred during the transit of manganese through the mitochondria, notably those of the liver.

Slow transportation of manganese may be induced readily by low intakes of this metal (19, 20, 25, 27). However, nutritional deficiency causes diminished tissue concentrations, whereas the arthritics had elevated concentrations of manganese in their red cells. It would have been interesting to ascertain whether they also had elevated manganese concentrations in the mitochondria of their livers. If the liver mitochondria tended to entrap this metal, other sites must have become manganese deficient. Such sites would include those that synthesized mucopolysaccharides: most steps of mucopolysaccharide synthesis proceed outside the mitochondria as well as outside of the liver (30). Furthermore, some critical biosynthetic steps require manganese (1–6).

Such a block would explain some of the abnormalities of mucopolysaccharide metabolism in rheumatoid arthritis (7–10). Of these, the one discovered by Laskin, Engel, Joseph, and Pollak (8) is intriguing because it proved reversible by the administration of glucocorticoid hormones.

The above proposal of manganese deficiency in the midst of plenty is merely a working hypothesis for further studies of rheumatoid arthritis. By contrast, the link between the action of prednisone and the distribution of manganese appears to be based upon fact. Earlier work with mice has shown that glucocorticoid hormones accelerate the distribution

and possibly the excretion of manganese (18, 19). Our present clinical study is in agreement with this finding, since prednisone accelerated the slow turnover encountered in arthritis, while it improved the disease. Therefore, it appears that a function of this hormone might be exerted by regulating the distribution of this and other transition metals.

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