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MANGANESE UPTAKE BY MOUSE LIVER SUBCELLULAR FRACTIONS. I. EFFECT OF FERRIC IONS

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SUMMARY

It has been shown that intraperitoneally injected ^{54}Mn concentrates primarily in mitochondrial and nuclear fractions of hepatic cells. The microsomal and the supernatant represented 59% and 42% respectively of the mitochondrial uptake. This pattern of distribution did not change with time and it was mantained for at least 5 days after injection. The *in vitro* studies revealed that 1 mM $FeCl_3$ produced a significant increase of ^{54}Mn uptake in each one of the subcellular fractions. It is believed that Fe^{3+} acts as oxidant in the following oxidation-reduction mechanism: 1) Mn^{2+} (dissociable) $-1e \longrightarrow Mn^{3+}$ (bound); 2) $Fe^{3+} + 1e \longrightarrow Fe^{2+}$.

INTRODUCTION

Manganese is preferentially distributed in association with the membranous fractions (mitochondria, nuclei, and microsomes) of rat (7, 10, 11) and human liver (11) and rat submaxillary glands (2). Radioisotopic studies (10) have revealed important information about the localization of ^{56}Mn (half life 2.59 hours) in intracellular organelles of liver before reabsorption and redistribution of the isotope took place.

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Iron and manganese are intimately related as suggested by their complementary subcellular distribution (7, 11): iron concentration is lowest in the nuclei and highest in the soluble fraction. Besides, in tissue iron deficiency an increased manganese absorption is produced. Conversely, tissue iron load is accompanied with decreased manganese absorption (6). Our **in vitro** assays evidenced that low Fe^{3+} concentrations (< 5 mM) added to mouse liver homogenates increased ^{54}Mn uptake but concentrations of Fe^{3+} as high as 10 mM abolished it (3).

This work was designed to determine the hepatic intracellular distribution of ^{54}Mn at regular intervals after its intraperitoneal injection so as to know to what extent the intestinal reabsorption and redistribution of the metal change the pattern of its subcellular localization. On the other hand, this study describes some characteristic effects of Fe^{3+} on ^{54}Mn uptake by the subcellular fractions of normal mouse liver.

MATERIAL AND METHODS

Adult male mice MRT strain (20-25 g) fed ad libitum with Purina Laboratory Chow (32 ug Mn/g) and distilled demineralized water were used in all experiments. For the **in vivo** assays two uCi of carrier free $^{54}MnCl_2$ (Amersham/Searle, Illinois) were injected intraperitoneally into each animal. After appropiate periods of time the mice were killed by cervical dislocation and the livers immediately removed, weighed and homogenized with a Teflon pestle in cold 0.25 M sucrose (pH 7.4) to provide a 10% homogenate. Subcellular fractionation was performed following the procedure of Hogeboom (8). Radioactivity on aliquots of each fraction was determined in a well-type scintillation counter (Packard Instruments Co., La Grange, Illinois). Protein was measured by the method of Lowry et al (9). Statistical analysis was performed using the Student's t test (1).

For the **in vitro** assays, aliquots of the fractions were incubated in buffer Tris/HCl 0.05 M, pH 7.4, with 0.02 uCi of $^{54}MnCl_2$. Reaction was initiated by addition of the fractions and the final volume was 1.0 ml. Incubation was performed for 10 min. at 37°C in a Dubnoff Metabolic Shaking Incubator (60 strokes per min) and the reaction stopped by the addition of 2.0 ml absolute ethanol. The tubes were centrifuged at room temperature in a Sorvall GLC-1 centrifuge at 1650 g X 15 min. The supernatant was discarded and the precipitate washed with 2.0 ml of the Tris/HCl buffer and centrifuged for 15 min. The washing procedure was repeated twice and the final precipitate counted for radioactivity. No complexing of Mn^{2+} by Tris has been previously demonstrated (12). In fact, in our hands no radioactivity was observed, when buffered $^{54}MnCl_2$ was incubated in the absence of the subcellular fractions, and the whole procedure repeated as described.

RESULTS

At all times about 85% of the radioactivity was located in cell organelles. As shown in table I the concentration of ^{54}Mn found in the homogenate one hour after the injection of the radioisotope, was lower than in the nuclear ($p < 0.002$) and mitochondrial ($p < 0.001$) fractions, similar to that of the microsomal fraction and higher than in the supernatant ($p < 0.05$). When compared, both the nuclear and the mitochondrial fractions reached similar ^{54}Mn concentration. In addition, although the total radioactivity was lower during the remainder time intervals studied, the intracellular distribution of ^{54}Mn did not differ from that previously described.

Under our experimental conditions 1.0 mM $FeCl_3$ produced a significant increase of ^{54}Mn uptake in each one of the subcellular fractions (Table II). In the presence of 1 mM ascorbate as a reducing agent the ^{54}Mn uptake was not affected. However, a significant decrease was produced by the addition of 10 mM ascorbic acid to the nuclear, microsomal and mitochondrial fractions. Adding $FeCl_3$ (1 mM) to the incubation medium in the presence of 1 mM ascorbic acid produced a significant increase in ^{54}Mn uptake by all the fractions, as compared with the controls, but the values obtained were lower than those produced by $FeCl_3$ (1 mM) in abscense of ascorbate. This was true for the nuclear ($p < 0.02$), mitochondrial ($p < 0.001$) and microsomal ($p < 0.002$) fractions. Ascorbic acid (10 mM), in the presence of Fe^{3+} (1 mM), caused a significant decrease in the ^{54}Mn uptake by all the subcellular fractions.

As shown in table III the utilization of the strong oxidazing agent, potassium persulfate ($K_2S_2O_8$), at the concentration of 10 mM, inhibited the ^{54}Mn uptake in the mitochondrial, microsomal, and supernatant although it did not alter the uptake in the nuclear fraction. At lower concentrations of $K_2S_2O_8$ the ^{54}Mn uptake of the subcellular fractions did not differ from the controls. When $K_2S_2O_8$ (0.01, 0.1, 1.0 and 10 mM) was assayed in the presence of $FeCl_3$ (1 mM) a significant increase in ^{54}Mn uptake was obtained in each one of the fractions as compared with controls free of added Fe^{3+}. The ^{54}Mn uptake in the presence of both 1 mM $FeCl_3$ and 1.0 mM or 10 mM $K_2S_2O_8$ was higher than the observed with $FeCl_3$ alone (Table IV). This behaviour was not seen with lower concentrations of $K_2S_2O_8$.

DISCUSSION

Maynard and Cotzias (10) have reported that intraperitoneally injected ^{56}Mn is rapidly distributed in rat tissues, concentrating primarily in organs rich in mitochondria. Liver cell fractionation studies revealed that the

TABLE I

SUBCELLULAR DISTRIBUTION OF ^{54}Mn IN MOUSE LIVER AT DIFFERENT TIME INTERVALS AFTER ITS ADMINISTRATION

Time after injection (h)	N° of Experiments	Homogenate	Subcellular Fractions			
			Nuclear	Mitochondrial	Microsomal	Supernatant
1	3	1549 ± 168	2290 ± 152	2474 ± 77	1452 ± 264	1040 ± 148
2	3	595 ± 39	1024 ± 109	932 ± 90	617 ± 62	478 ± 64
8	2	558 ± 12	976 ± 90	952 ± 82	530 ± 47	276 ± 36
24	1	407	712	695	387	201
48	1	365	638	622	347	180
96	1	279	480	476	265	140
120	1	212	370	362	200	90

Two μCi of carrier free $^{54}MnCl_2$ were injected intraperitoneally into each animal. After appropiate periods of time the mice were killed and their livers homogenized in 0.25 M sucrose, pH 7.4 to provide a 10% homogenate. After fractionation, the radioactivity on aliquots of each fraction was determined in a well-type scintillation counter. Results are expressed as cpm per mg of protein ± S.E.M.

TABLE II

IN VITRO ^{54}Mn UPTAKE BY MOUSE LIVER SUBCELLULAR FRACTIONS.
EFFECT OF $FeCl_3$ AND ASCORBIC ACID ADDED TO THE INCUBATION MEDIUM.

Concentration of Compounds	Subcellular Fractions		
	Nuclear	Mitochondrial	Microsomal
Standard incubation medium	100 ± 00	100 ± 00	100 ± 00
1 mM $FeCl_3$ (4)	431 ± 46*	267 ± 18*	309 ± 40*
1 mM Ascorbic Acid (3)	123 ± 24+	114 ± 18+	108 ± 19+
10 mM Ascorbic Acid (2)	30 ± 8*	23 ± 3*	37 ± 5*
1 mM $FeCl_3$ + 1 mM Ascorbic Acid (4)	284 ± 37*	145 ± 10*	205 ± 17*
1 mM $FeCl_3$ + 10 mM Ascorbic Acid (3)	22 ± 2*	18 ± 2*	24 ± 3*

Standard incubation mixtures (1.0 ml) contained the subcellular fractions suspended in 0.25 M sucrose, 0.02 uCi $^{54}MnCl_2$ and buffer Tris/HCl 0.05M, pH 7.4 The reaction was initiated by addition of the subcellular fractions. Incubation was for 10 min at 37°C. The ^{54}Mn uptake was expressed as mean percentage of control values ± S.E.M. Figures in brackets represent number of experiments carried out by triplicate.

* $p < 0.001$, when compared with controls. +No significative.

TABLE III

IN VITRO ^{54}Mn UPTAKE BY MOUSE LIVER SUBCELLULAR FRACTIONS. EFFECT OF $K_2S_2O_8$.

Concentration of Compounds	Subcellular Fractions			
	Nuclear	Mitochondrial	Microsomal	Supernatant
1 mM $FeCl_3$ (4)	431.0 ± 46.0*	267.0 ± 18.0*	309.0 ± 40.0*	585.5 ± 129.0*
10 mM $K_2S_2O_8$ (4)	116.8 ± 38.8+	64.3 ± 6.7*	64.7 ± 16.4**	57.2 ± 7.4*
1 mM $K_2S_2O_8$ (9)	166.0 ± 41.7+	80.3 ± 15.9+	107.9 ± 15.3+	118.9 ± 13.9+
0.1 mM $K_2S_2O_8$ (6)	190.5 ± 75.6+	121.6 ± 23.8+	132.2 ± 28.0+	122.0 ± 23.1+

Figures in brackets represent number of experiments carried out by triplicate. The uptake was expressed as mean percentage of control values ± S.E.M.

*: $p < 0.001$ compared to control values. **: $p < 0.05$ +: No significative.

TABLE IV

INCREASED ^{54}Mn UPTAKE PRODUCED BY $FeCl_3$ IN THE PRESENCE OF $K_2S_2O_8$ IN THE INCUBATION MEDIUM

Concentration of Compounds	Subcellular Fractions			
	Nuclear	Mitochondrial	Microsomal	Supernatant
1 mM $FeCl_3$ (5)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
1 mM $FeCl_3$ + 10 mM $K_2S_2O_8$ (5)	152.7 ± 24.0^{+}	191.5 ± 23.2*	134.9 ± 11.5**	117.8 ± 5.1*
1 mM $FeCl_3$ + 1 mM $K_2S_2O_8$ (5)	116.3 ± 2.8*	134.5 ± 7.6*	114.4 ± 2.7*	114.3 ± 6.3*
1 mM $FeCl_3$ + 0.1 mM $K_2S_2O_8$ (5)	100.1 ± 1.55^{++}	99.9 ± 5.5^{++}	98.8 ± 4.2^{++}	107.2 ± 5.8^{++}
1 mM $FeCl_3$ + 0.01 mM $K_2S_2O_8$ (5)	98.4 ± 3.9^{++}	99.5 ± 3^{++}	98.4 ± 7.5^{++}	110.5 ± 6.8^{++}

Results expressed as mean percentage of 1 mM $FeCl_3$ values ± S.E.M. Figures in brackets represent the number of experiments carried out by triplicate.

*: $p < 0.001$ compared to 1 mM $FeCl_3$ values. **: $p < 0.005$. $^{+}$: $p < 0.05$. $^{++}$ No significative.

uptake of the isotope by mitochondria was twice that of the unfractionated homogenate while in the remainder fractions the uptake was lower. They concluded that the distribution of manganese is compatible with is functioning as a respiratory cofactor. These **in vivo** studies on the kinetics of manganese in subcellular fractions were performed with the short half life ^{56}Mn which can give information about the early portion of the distribution process. Our work with ^{54}Mn (half life 310 days) showed that although hepatic mitochondrial and nuclear fractions demonstrated the highest uptake, the values for microsomal and supernatant were also significative. In fact, their uptake represented about 59% and 42% respectively of the mitochondrial uptake observed 1 hour after ^{54}Mn injection. On the other hand, it was evident that this pattern of distribution did not change with time in spite of the reabsorption, redistribution, and the displacement of radiomanganese by dietary stable manganese (4).

This work evidenced that 1 mM Fe^{3+} increased ^{54}Mn uptake in all the subcellular fractions. It is known that in the protein-manganese complexes the valence state of manganese is Mn^{3+} (5). Our **in vitro** experiments in the presence of 1 mM Fe^{3+} seem to suggest that the following oxidation-reduction mechanisms are operative in the binding of Mn^{3+} by liver subcellular fractions.

1) Mn^{2+} (dissociable) $- 1e \longrightarrow Mn^{3+}$ (bound)
2) $Fe^{3+} + 1e \longrightarrow Fe^{2+}$

In this case Fe^{3+} acts as an oxidizing agent. Then, any reducing agent will tend to break the complex. As judged by the results obtained with 10 mM ascorbic acid this supposition looks true. Although 1 mM ascorbic acid did not affect ^{54}Mn uptake, in the absence of added Fe^{3+}, when it was assayed in the presence of Fe^{3+} it reduced the activation that is normally produced by the latter.

When 10 mM $K_2S_2O_8$ was used as oxidizing agent a decrease in ^{54}Mn uptake was obtained due probably to a full oxidation to Mn^{4+} or to higher oxidation numbers which do not form protein-metal complexes. However, the increase activation in ^{54}Mn uptake produced by $K_2S_2O_8$ (1 mM and 10 mM) in the presence of Fe^{3+} (1 mM) could be possibly explained by a continuos re-oxidation of Fe^{2+} to Fe^{3+} as follows:

1) $Mn^{2+} - 1e \longrightarrow Mn^{3+}$
2) $Fe^{3+} + 1e \longrightarrow Fe^{2+}$
3) $Fe^{2+} \xrightarrow[-1e]{K_2S_2O_8} Fe^{3+}$

Fe^{3+} would then be used to oxidize Mn^{2+} to Mn^{3+}.

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RESUMEN

Captación de manganeso por las fracciones subcelulares de hígado de ratón. I. Efecto del ión férrico. *Bonilla E. (Instituto de Investigación Clínica, Apartado 1151, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela).* **Invest Clín** *19(1): 31-40, 1978.*– Se ha demostrado que el Mn^{54} inyectado por vía intraperitoneal se concentra principalmente en las fracciones mitocondrial y nuclear de las células hepáticas. La fracción microsomal y el sobrenadante representaron el 59% y el 42%, respectivamente, de la captación mitocondrial. Esta forma de distribución no cambió con el transcurso del tiempo y se mantuvo por lo menos durante 5 días después de la inyección. Los estudios **in vitro** revelaron que 1 mM $FeCl_3$ produjo un incremento significativo en la captación de Mn^{54} en cada una de las fracciones subcelulares. Creemos que el Fe^{3+} actúa como oxidante en el siguiente mecanismo de óxido-reducción:

1) Mn^{2+} (disociable) $- 1e \longrightarrow Mn^{3+}$ (fijado)

2) $Fe^{3+} + 1e \longrightarrow Fe^{2+}$

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