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Manganese and Hydralazine as Studied by Neutron Activation and Radioactive Mn. (31426)

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Hydralazine produces a "Lupus-like" syndrome in a few patients. This syndrome has been reproduced experimentally in the laboratory, and, circumstantially, a Mn-deficiency seemed implicated. The results of a dietary deficiency of Mn were compared to the effects of hydralazine administration. The alterations produced by dietary Mn found in mice were not duplicated by hydralazine.

Studies relating hydralazine ("Apresoline," CIBA) to manganese (Mn) metabolism have suggested that a Mn deficiency might be produced by long term administration of this drug(1). Because hydralazine can induce a syndrome similar to Systemic Lupus Erythematosus (SLE) in patients, it seemed possible that Mn deficiency might play a role in the etiology of this disease.

As neutron activation analysis (NAA) has proven to be a specific and accurate technique to measure tissue concentrations of Mn, it was used to determine whether hydralazine administration might lower Mn in tissues. Although tracer techniques are not a perfect answer to the study of physiologic disposition and metabolism of a biological material, the apparent identity of a compound and its radioisotope from a physiological point of view is widely accepted. Thus, kinetic studies utilizing ^{54}Mn were performed to determine whether hydralazine might induce functional deficiency of this trace metal.

The results of hydralazine administration were compared with the alterations of Mn induced by dietary manipulations of Mn. Tissue concentrations of Mn, whole body turnover rates of ^{54}Mn, and organ partition studies of ^{54}Mn responded readily to dietary alterations of Mn but not to hydralazine administration.

Male Swiss albino mice (BNL strain) were used throughout these studies. Effects of hydralazine on tissue levels of Mn were compared with effects of Mn-deficiency-inducing diet, as described by Hurley and associates (2). This diet contained 40 μg/g of Mn (unpublished data). ^{54}Mn kinetics were studied in mice fed a low Mn diet as well as in mice on a high Mn diet. Low Mn diets* contained

* Low Mn diet supplied by Nutritional Biochem-

TABLE I. Mn Content of Mouse Tissue.

Tissue	Regime	No. of samples	μg/g (mean) wet wt	± S.D.	t	p
Liver	Hydralazine	12	.99	.07	.04	>.45
	Control	12	1.01	.11		
	Deficiency	10	.21	.04	15.7	<.005
	Control	10	1.21	.12		
Kidney	Hydralazine	12	1.23	.15	1.67	>.05
	Control	12	1.28	.11		
	Deficiency	10	.45	.08	76.5	<.005
	Control	10	1.35	.07		
Brain	Hydralazine	12	.33	.03	1.49	>.05
	Control	12	.35	.04		
	Deficiency	10	.27	.03	10.3	<.005
	Control	10	.38	.04		

1.8 μg/g of Mn, as determined by NAA (unpublished data). The high Mn diet was exactly the same as the low Mn diet except that 0.55 mg of Mn per ml was added to the drinking water.

Hydralazine was administered subcutaneously 1.5 mg/day (corresponding to a dose of 100 mg/kg) 6 days per week. Tissue levels of manganese were determined after 6 and 8 weeks of injections. NAA revealed hydralazine as injected contained 0.5 μg/l.* Carrier-free $^{54}MnCl_2$ 1/2 microCi was injected intraperitoneally or subcutaneously and radioactivity determined with a NaI (T1) crystal and a single channel gamma ray spectrometer. In ^{54}Mn distribution studies, "carcass" is defined as skin, muscle, skeleton, central nervous system, and their combined connective tissue, *i.e.*, it excludes G.I. tract and contents, kidneys, liver, spleen, heart, lungs, and testicles. "C/L ratio" is defined as the ratio of ^{54}Mn radioactivity of the "carcass" divided by the ^{54}Mn radioactivity of the liver. NAA was performed* as described previously(3).

Samples of tissues from liver, kidney, diaphragm, and brain from mice treated with hydralazine contained the same amount of stable manganese as control mice, whereas the same tissues for mice on an Mn-deficiency-inducing diet contained a markedly lower concentration of Mn as compared to tissues of control mice (P values of the latter were all <0.005) (Table I).

In Fig. 1, the whole body turnover rates of ^{54}Mn in mice injected with hydralazine are compared to the rates of control-injected mice, and the turnover rates in mice on an Mn-deficiency-inducing diet are compared to that of control mice (note the difference in time scale). The markedly decreased turnover rate produced by the Mn-deficiency-inducing diet is obvious, as is the only minimally in-

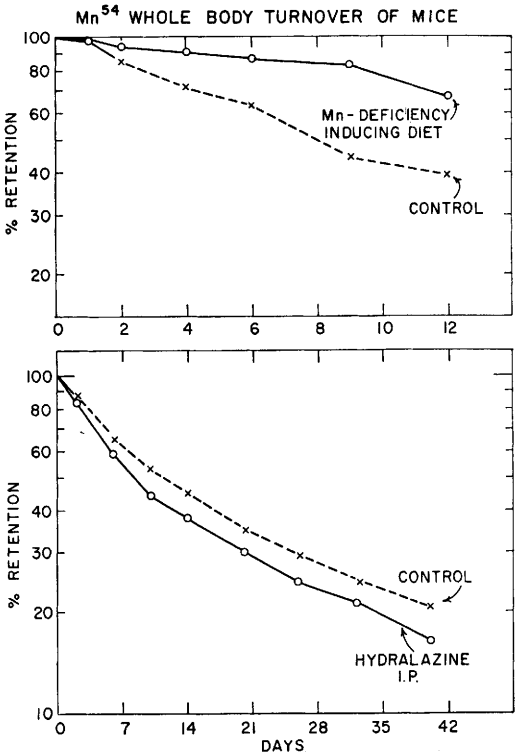

FIG. 1. Whole body turnover of mice.

icals Co.; $^{54}MnCl_2$ supplied by Nuclear Science & Engineering Corp.; NAA performed through the courtesy of J. M. Edwards.

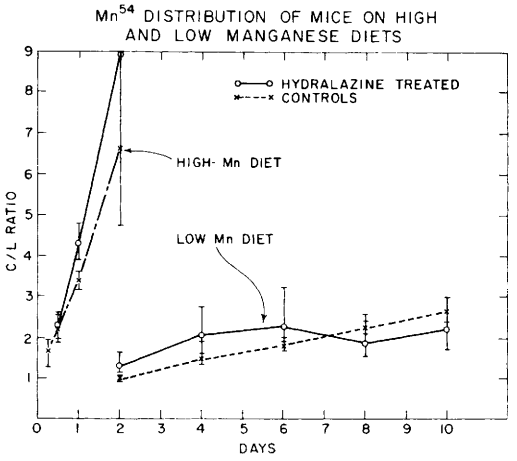

FIG. 2. Mn^{54} distribution of mice on high and low manganese diets.

creased turnover rate of whole body ^{54}Mn of hydralazine administered mice. In Fig. 2 are plotted the C/L ratios of mice against time, where zero is the time of injection of isotope and the beginning of daily injections of hydralazine. The organ partition studies as typified by the C/L ratios disclose that hydralazine administration has no significant effect whereas dietary manganese has significantly altered the rate of change of this ratio.

Through the use of NAA, it was demonstrated that tissue levels of Mn were not altered by hydralazine administration. However, tissue levels of mice on an Mn-deficiency-inducing diet were markedly diminished. In addition, tracer studies utilizing ^{54}Mn revealed that profound alterations of both turnover rates and organ partition ratios were produced by the deficiency-inducing diet but not produced by hydralazine administration. Thus, alterations of dietary Mn produced tissue concentration changes of stable Mn and kinetic changes of ^{54}Mn. On the contrary, hydralazine administration produced none of these characteristic changes.

It has been amply demonstrated that hydralazine and Mn interact. Convulsion induced by hydralazine in rats can be specifically prevented by parenteral Mn(1,4). This has been duplicated in mice (unpublished data). Hydralazine can inhibit the dramatic organ partition changes (a rise of the C/L ratio of ^{54}Mn in mice) induced by hydrocortisone(5). Comens has demonstrated that some of the signs of SLE induced in dogs by hydralazine administration can be ameliorated by supplements of Mn orally. However, the results presented here suggest that the phenomenon presented by Comens cannot be explained by a Mn deficiency. Obviously, further work will be needed to clarify the interrelationship of hydralazine, Mn, and SLE.

In this study, the combination of NAA and tracer techniques has demonstrated that dietary Mn produces specific alterations of Mn tissue concentrations and Mn kinetics. Also, it has permitted a comparison of the effects of hydralazine administration to Mn deficiency.

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