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MANGANESE POISONING - NEW INSIGHTS *

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INTRODUCTION

The heavy metal intoxications involving the brain may be divided into two groups: One, in which the offending metal is clearly a poison, such as mercury or lead; and a second, in which the offending agent is normally an essential constituent of the body. The latter category would include manganese poisoning, copper poisoning (or Wilson's disease) and, if some recent data are valid, Huntington's chorea, in which iron seems to have been implicated.

Our interest in the second category of diseases is due to the fact that these conditions represent special aberrations of normal homeostasis. Homeostasis is the sum of the processes which maintain constant structure, function and constitution in the body and its organs. In manganese poisoning, the homeostatic control is disturbed by virtue of oversupply of this element *via* an abnormal route (the respiratory tract), while in Wilson's disease homeostasis is disturbed by virtue of a genitically transmitted abnormal avidity of the body for dietary copper.

In the present paper, we will discuss first some of the clinical features of chronic manganese poisoning in order to raise some questions about extrapyramidal syndromes in general. Thereafter, we will allude to some investigations conducted at this Center during the last ten years. While these studies pertained to the normal metabolism of manganese, the techniques developed for this work also apply to researches on human manganese poisoning. Some observations arising from the treatment of Wilson's disease will be discussed in connection with manganese poisoning. Finally, the "poisonous metals" will be compared with the "essential" ones in

relation to the induction of extrapyramidal dysfunctions. It will be proposed that the melanin granules of the extrapyramidal system might be a common site of action of many of these metals.

Clinical Features of Manganese Poisoning:

This is a disease of insidious onset, characterized by progressive mental and/or extrapyramidal manifestations. It occurs primarily in industries such as mining, in which workers are exposed to inhalation of manganic ore dusts. Only some of the exposed workers develop this condition indicating an individual susceptibility, possibly of the kind alluded to in relation to the induction of Parkinsonism by phenothiazine derivatives (1).

The incidence of this disease is fairly high throughout the world. It occurs often in epidemic outbreaks. The existance of cases of manganese poisoning in Chile is particularly noteworthy: These patients are located in the proximity of modern, fully equipped, and well staffed medical centers, in which the tradition for research is well established. Hence it appears that the conditions for extensive study of this disease are flourishing in that country.

The pathogenesis of manganese poisoning has not been studied fully. The precise mechanism of damage is a matter of further work. Of possible pertinence in such studies are the route of administration (parenteral); the chemical form of manganese (manganic) and the size of the particle ($< 5\mu$ (2)).

If animal work is to be considered relative to the pathogenesis of manganese poisoning, one might note that several workers, (including

Dr. Pentchew, who is here today) have studied laboratory models of this disease. It is noteworthy that neurological signs have been observed only in higher animals, while the lesions to which these signs have been ascribed can be induced apparently in some lower animals also. These lower animals however, remain free from neurological manifestations, in spite of their brain lesions. This discrepancy causes concern with regard to whether the pathological findings are solely responsible for the functional abberations of the brain ascribed to them. One's concern is heightened by the fact that in Wilson's disease also there might occur a possible dissociation between functional aberrations and pathological lesions: the former tend to subside following removal of copper from the patient's body and from his diet. Does the well known lack of regenerative potential of the brain permit us to believe that the lenticular degeneration tends to subside also? It would be interesting to see whether a parallel experience might occur after treating manganese poisoning (3).

The prodromal phase of manganese poisoning is often similar to an encephalitis, since somnolence, insomnia, headache and leucopenia often occur. During this phase, the patient might commit acts of violence, sometimes involving sexual offenses. Confusion, delirium and other mental manifestations have been describedas well.

The extrapyramidal phase of this disease might either overlap with the previously described clinical picture or each might occur separately. Clumsy articulation and mask-like facies may appear. Muteness has sometimes been observed. Rigidity with cog-wheel phenomenon is often present, as is pes striatum. A tremor is often seen and, when present, becomes exaggerated by emotional stress, fatigue or trauma. Crying spells interrupted by

laughter have been described. Loss of associated movements and disturbances of propulsion and gait are also present. Autonomic disturbances such as excessive salivation and excessive sweating may become apparent. Pyramidal signs are reported as lacking.

A distinct form of manganese poisoning has been described in which marked rigidity or painful spasms of the musculature prevail, These patients seem to assume bizarre postures and exhibit "Hahnenschritt" and pes striatum.

This clinical description is based on several published reports (2, 3, 4, 5, 6). The prevalence of the various symptoms seems to differ in different countries as well as in different outbreaks of the disease. Hence no attempt has been made here to present them in the order of their frequency.

A manganese pneumonopathy often accompanies chronic manganese poisoning of the brain. Since however, the lungs may be afflicted independently of the brain, the pneumonopathy is not a necessary prerequisite in the development of the extrapyramidal syndrome (7).

The diagnosis of this disease rests on the knowledge of the patient's occupation as well as of current outbreaks of manganese poisoning. A positive permanganate color produced by oxidizing properly collected samples of urine is said to be pathogenomonic of this disease. The clinical picture on the other hand,can be variable as was previously stated. This is demonstrated in the following moving picture.

This moving picture shows two North American patients from our Hospital being compared to two Chilean mineros, whom we photographed in La Serena, Chile. The differences among these patients are not as

impressive, to our minds, as are their similarities, since the North Americans have Wilson's disease and Parkinson's disease respectively, while the two South Americans suffer from manganese poisoning. Such similarities among patients having diseases of entirely different etiologies might argue for a substratum common to all of them. This will be discussed below.

STUDIES ON THE METABOLISM OF MANGANESE

Techniques:

The manner in which the observations which follow were consummated cannot be given in detail. Hence, only some cardinal techniques will be touched upon. Furthermore, the continuity of this discussion necessitated some rearrangement of the chronogical sequence at which these observations were published originally.

Modern studies of manganese metabolism require that one can define at least three characteristics of this metal in the intact organism or in samples therefrom: 1) The metal's quantity. 2) Its movements into, within and out of the body. 3) Its physiochemical state. In our Center, the first is being assessed by neutron activation analysis, the second is studied with the aid of isotopes and the third is investigated with electron spin resonance, which is a magnetic technique.

From the medical point of view, quantitation of this metal is important only if it applies to samples which are readily obtainable by a physician. Such samples are urine, blood, plasma, cerebrospinal fluid, bile, etc. These body fluids are poor in manganese, since they contain about one hundredth to one thousandth of the metal's concentration as compared to solid tissues. Even in solid tissues, however, manganese is a trace element as can be seen from Slide 1, which shows the total body

concentration of various essential elements for a 70 kg. man. Since the ordinate is logarithmic, this slide makes it amply evident that one has to use special techniques if one wants to measure the tiny concentration of this element in clinical samples. It should be noted that with some earlier techniques, large amounts of blood were necessary for a single analysis. Even so contaminations and losses were frequent. These complications are preventable by the use of a highly sensitive technique, namely neutron activation analysis.

In neutron activation analysis one exposes a sample (i.e. blood, plasma, etc.) to neutrons produced by a nuclear reactor. The interaction of the neutrons with the manganese of tissues renders this metal radioactive. The radioactivity is proportional to the amount of manganese present in the sample. By measuring this radioactivity and comparing it to that of a similarly treated standard consisting of pure manganese, one can compute the concentration of this element in the original sample. This method yields accurate results with 0.2 ml samples of plasma (8 0).

With neutron activation analysis, one measures the quantity of natural manganese by rendering the metal radioactive. For the "kinetic" type of information however, we have relied on artificial manganese radioisotopes which we have been introducing into animals and man by feeding or by injection. The radioisotopic techniques have shown not only some sites of preferential localization of manganese, but also the rates of speed at which these localizations occur. Furthermore, the use of radioactivity measurements of the entire body has tended to supplant some of the balance studies practiced earlier in such metabolic investigations.

OBSERVATIONS

The concentration of manganese was determined in the blood, plasma, serum and cerebrospinal fluid of either hospitalized patients or some laboratory workers. The number of patients, the mean manganese value (μg/liter) and the standard deviation from the mean are given below:

8 serum samples:	2.46 ± 0.30
20 plasma samples:	2.22 ± 0.66
13 whole blood samples:	10.45 ± 2.27
6 cerebrospinal fluid samples:	1.18 ± 0.23

Although these patients were unselected and only the conditions of sampling were standardized, the values indicated above were remarkable in two respects: First, the amount of manganese in these samples was tiny indeed. Second, tiny though it was, it was of about the same magnitude from person to person. This indicated to us that there might exist homeostatic controls for this element's concentration within the body pools sampled for analysis.

In view of the well-known incorporation of plasma copper into ceralloplasmin, the question arises whether manganese is similarly incorporated into some plasma protein. The copper in cerulloplasmin is tightly bound to the protein molecule and cannot be dissociated from it either completely or readily (9). In contradistinction to copper, manganese was found to be loosely bound to plasma proteins, since, although approximately 90-96% of the metal was protein-bound, almost all of it could be dissociated by acidification of the plasma (10). These results pertained both to the native plasma manganese, as well as to the radioactive isotope (Mn^{54++}) which had been added to plasma <u>in vitro</u>. Furthermore, on electrophoresis

the bulk of the protein bound element seemed attached to the β_1 globulin fraction and only small amounts to α globulin and to albumin (11).

In contradistinction to plasma, the red cell bound manganese was found to be almost non-dissociable by acidification, while the element found in spinal fluid was almost totally dissociated from proteins. The first of these findings was in keeping with the earlier claim that manganese becomes incorporated into the heme moiety of hemoglobin, most probably in the form of a manganoporphyrin (12). This finding might have some bearing on manganese poisoning.

The steady concentrations reflected in the above analyses might be relevant to the fact that the kinetic behavior of this element in mice (13) and in humans (14, 15) is sensitive to the action of glucocorticoid hormones. These hormones are instrumental in homeostasis. Treatment of either animals or patients with these agents has resulted in two types of alteration of the metabolism of this element. First, the internal distribution and the rate of excretion of parenterally administered radiomanganese (Mn^{54}) became accelerated as a consequence of treatment. Second, the plasma level of this element became elevated following administration of cortisone analogues. Slides 2 and 3 illustrate these results. Whether these effects are necessary for the therapeutic action of these drugs is a matter of conjecture for the moment. It is conceivable however, that prolonged treatment with these agents, as is being practiced in cases of collagen disease, aplastic anemia, etc., might result in a manganese deficiency, parallel to the well-known potassium deficit which accompanies such treatment.

A distortion of the rate of excretion and of the blood concentration of manganese was also studied as a consequence of feeding large amounts of manganous sulfate (2.0 gm $MnSO_4$ per day) to a patient (Hospital #7826R). The rate of loss of radiomanganese from the liver of this patient as well as from the entire body was markedly accelerated by the feeding of the manganese salt (Slide 4). Concommitantly, the blood and cerebrospinal fluid levels of the natural manganese became abnormally high during the administration of $MnSO_4$. The blood manganese became quadrupled and the cerebrospinal fluid concentration became doubled. These values tended to regress after this administration was discontinued. These observations indicated that one might expect similar distortions in cases suffering from chronic manganese poisoning, in spite of some earlier evidence to the contrary (5).

Furthermore, this same observation focused our attention on the role of the liver in the metabolism of this element. In earlier experiments,involving rats (16), it was found that the liver and the pancreas of these animals constituted organs of high accumulation of parenterally injected radiomanganese (Mn^{56}). Furthermore, in the liver at least, this element had become localized primarily in the mitochondria of this organ, an observation which was confirmed in the mouse also (Slide 5). Indeed a proposal was made to utilize the clearance of radiomanganese from the bloodstream as a possible measure of mitochondrial function in the intact man (17).

The observations alluded to above are pertinent also to the regulation of the excretion of manganese. This element is excreted almost totally into the gastrointestinal canal. The main excretory routes seem to be <u>via</u> the liver and <u>via</u> the pancreas, which had been mentioned earlier as being loci in which this element accumulates. In a communication

recently submitted for publication (18), some experiments were presented which indicated that the biliary route regulates the flow of manganese out of the body in response to varying dietary supply. The pancreas, which is also capable of excreting this element, can vary the element's flow only when extraordinary loads of metal are imposed on the body. These findings suggest that the liver and the pancreas must be studied in relation to aberrations that might accompany chronic manganese poisoning. Among the various functions of these organs, the one's reflecting primarily the mitochondrial activity might be germane.

For reasons which are not pertinent to the present discussion, but which are detailed elsewhere (14, 15, 19, 20) it had become important for us to study the concentration of manganese in various melanin containing structures. Analyses of human hair showed that the concentration of manganese varied from one person to another, and this variance was seen in animals also. Nonetheless, when dark hair was compared to light hair from the same individual, the concentration in the former was found always to be at least double of that in the latter. This difference was equally striking when barbs from multicolored feathers were analyzed for manganese. These results indicated that, in non-viable structures, the pigment granules must contain high amounts of manganese. A viable structure, namely the bovine conjunctiva showed identical findings: Its pigmented portions contained more than twice the amount of manganese found in the unpigmented segments.

This encouraged us to believe that possibly all melanin granules might be loci of preferential accumulation of this element. Hence, we turned to an animal, the liver of which is rich in melanocytes. This animal is the eel Amphiuma, on which we are testing some of the hypotheses

with which this article will conclude. The isolated melanin granules of Amphiuma liver had higher concentrations of manganese per unit weight of granules than did the original liver. On looking up the literature (21-26) we found that such granules had been known to contain many other metals as well. Zinc, copper, and iron are the main metallic constituents of these granules, but other metals have been localized there also. The presence of such metals in these melanin granules, reminded us of a cardinal rule of coordination chemistry, which, simply phrased, states that receptors which have avidity for one metal will have some avidity for other metals also. It is well known that the extrapyramidal system contains melanin granules in the substantia nigra and in the locus coeruleus. It is equally well known that manganese, copper and lead may induce extrapyramidal dysfunctions in man. The question arose therefore whether the melanin granules in the extrapyramidal system might not reflect a site of action common to all these metals.

In a publication which will appear shortly (27) we are presenting supporting evidence for a correlation between various extrapyramidal dysfunctions and the state of melanogenesis in the brain. The latter can be defined only grossly with histological techniques. Even so, at least two conditions characterized by extrapyramidal manifestations show loss of pigment granules from the substantia nigra: Parkinson's disease and phenylpyruvic oligophrenia. Furthermore, the monkey which develops both lesions and clinical signs upon exposure to manganic salt does have pigment in its substantia nigra; while the rat, which develops only lesions but no clinical signs, does not have pigment in that area (28 - 30). With more sensitive criteria one expects to encounter many more correlations between melanogenesis in the brain and various extrapyramidal disorders, as is being

discussed in the aforementioned article (27). For the moment, suffice it to state that the sum of the technical and physiological information presented here seems applicable to a model of extrapyramidal diseases, namely manganese poisoning.

SUMMARY

Manganese poisoning was discussed as being a clinical model in the study of extrapyramidal disorders. Various technical developments were mentioned which have extended our capabilities for investigating the metabolism of manganese. Some observations were presented on the normal physiology of this element and on some distortions thereof. The high concentration of manganese in melanin granules was noted. These bodies were proposed as possibly reflecting chemical and physical changes underlying some extrapyramidal diseases including manganese poisoning.

LEGENDS TO FIGURES

Figure 1 — Computed from: Altman, P.L. (ed.) BLOOD AND OTHER BODY FLUIDS, Fed. of American Societies for Experimental Biology (1961): Spencer, William S. (ed.) HANDBOOK OF BIOLOGICAL DATA, W. B. Saunders Co., Phila., Pa. (1956); and Tipton, I. H., Cook, M.J. Health Physics, 9, 103 (1963).

Figure 2 — Change of the Mn^{54} concentration in the whole body, the liver and the mid-thigh of a woman receiving prednisone.

Figure 3 — Same as Figure 2, but with plasma manganese concentration plotted.

Figure 4 — By permission, J.B. Lippincott Company, Philadelphia (see text).

Figure 5 — Intracellular distribution of Mn^{56} recalculated from Reference No. 16.

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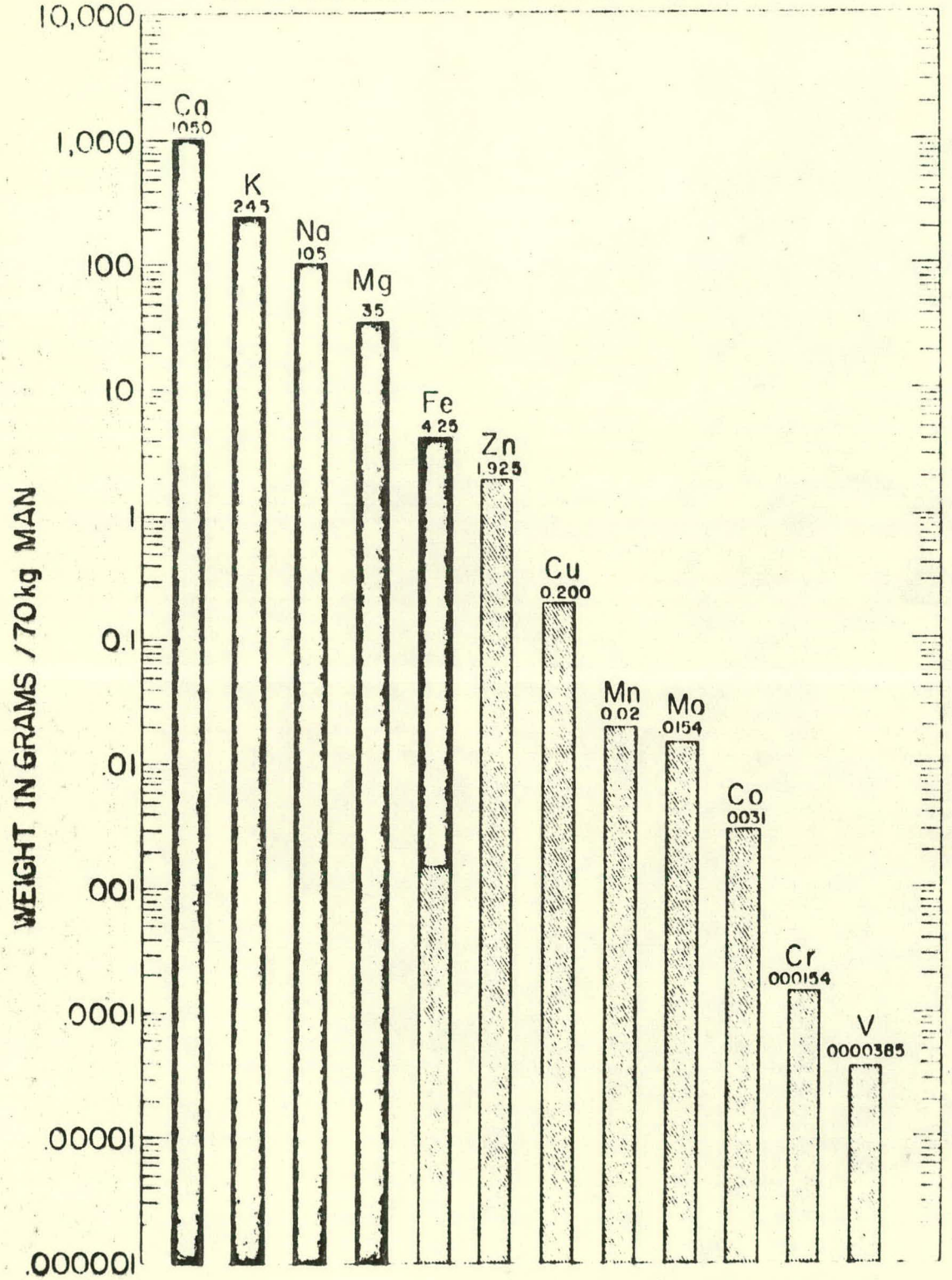
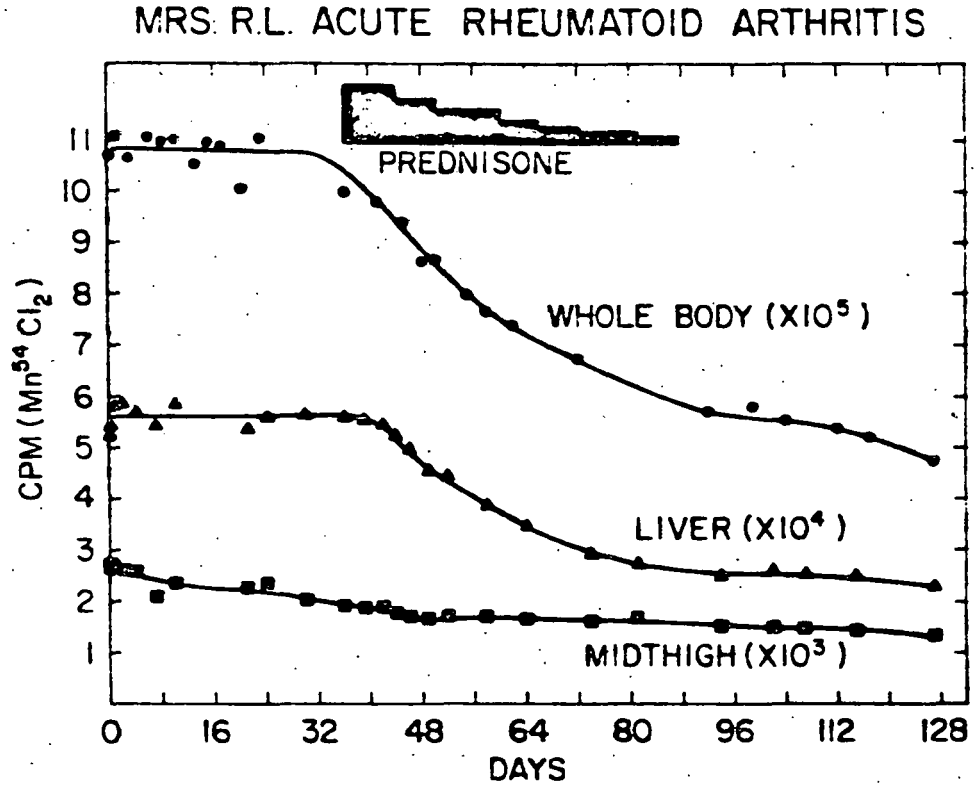
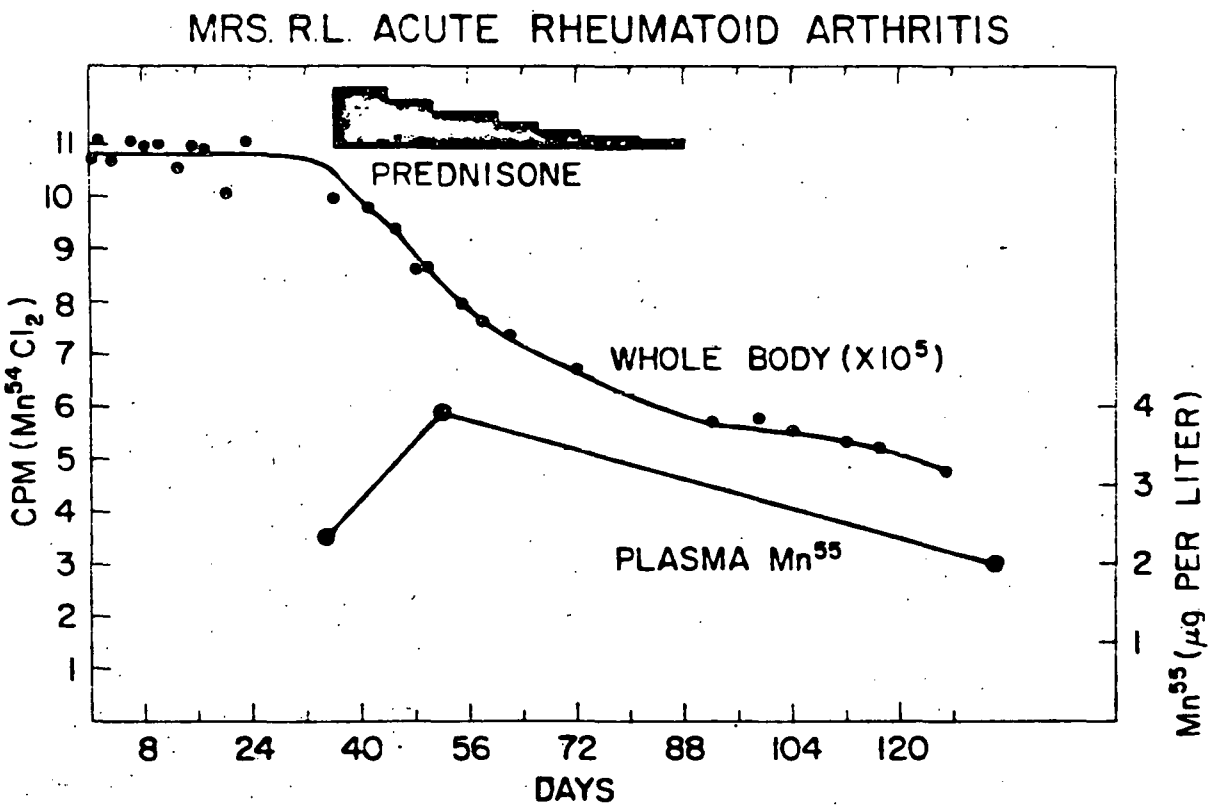
BEST ESTIMATES OF ESSENTIAL METALS IN MAN



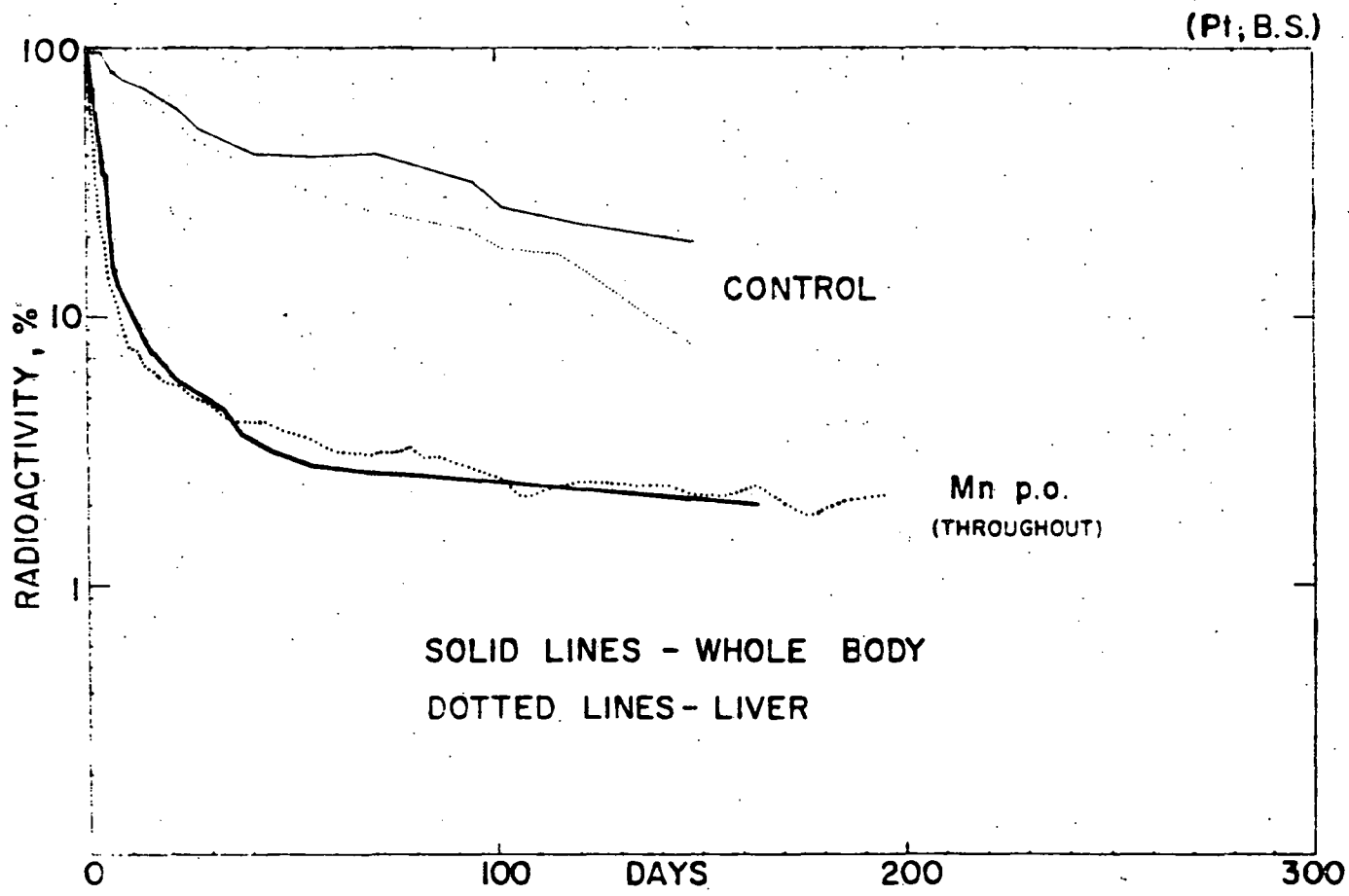
SLIDE I


MRS. R.L. ACUTE RHEUMATOID ARTHRITIS
PREDNISONE
WHOLE BODY (X10⁵)
LIVER (X10⁴)
MIDTHIGH (X10³)
CPM (Mn⁵⁴ Cl₂)
11
10
9
8
7
6
5
4
3
2
1
0
16
32
48
64
80
96
112
128
DAYS

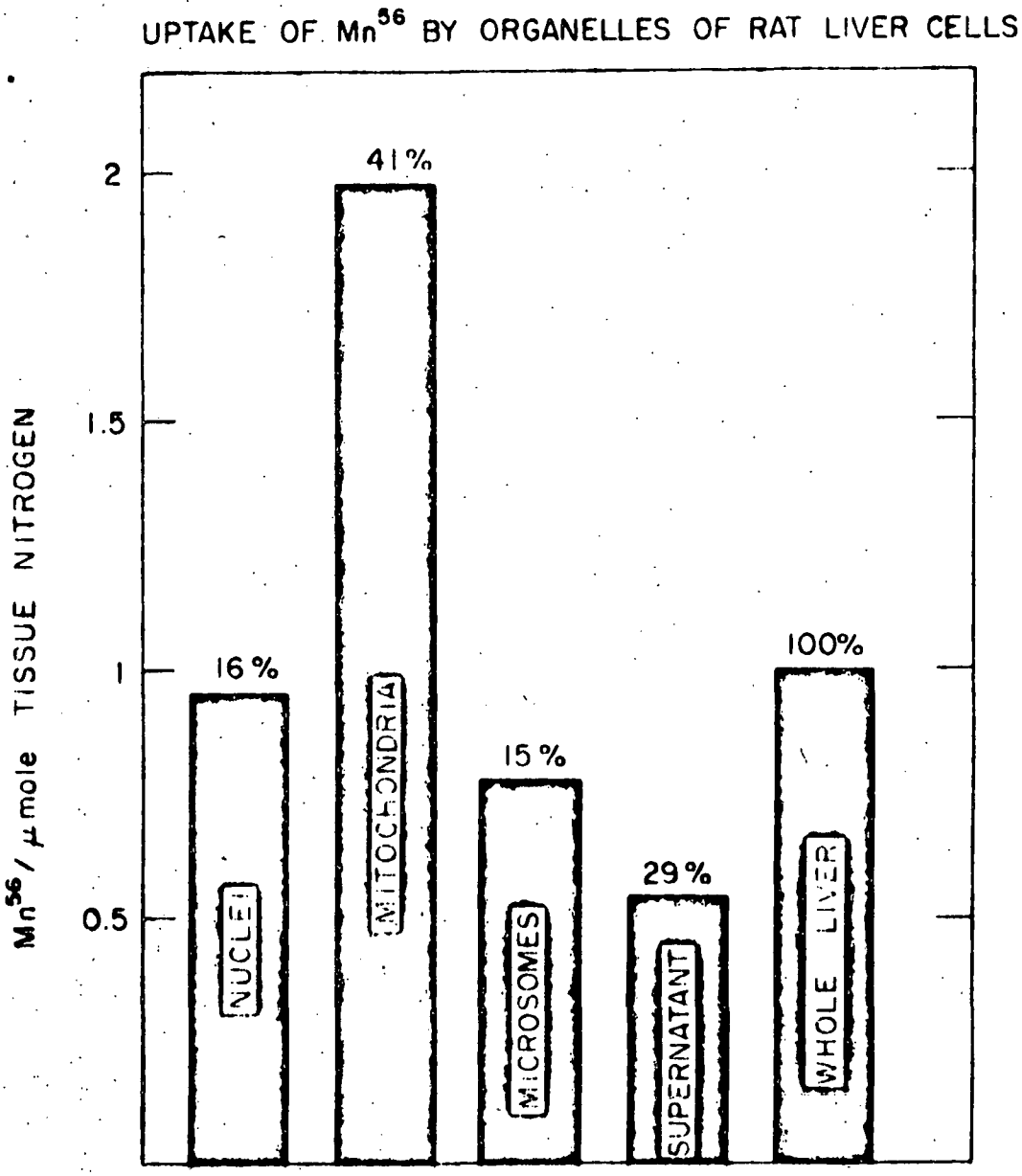
SLIDE II


MRS. R.L. ACUTE RHEUMATOID ARTHRITIS
PREDNISONE
WHOLE BODY (X10⁵)
PLASMA Mn⁵⁵
CPM (Mn⁵⁴Cl₂)
Mn⁵⁵ (μg PER LITER)
DAYS
8
24
40
56
72
88
104
120

SLIDE III


(Pt; B.S.)
100
10
1
RADIOACTIVITY, %
CONTROL
Mn p.o.
(THROUGHOUT)
SOLID LINES - WHOLE BODY
DOTTED LINES - LIVER
0
100
DAYS
200
300

SLIDE IV


UPTAKE OF Mn^{56} BY ORGANELLES OF RAT LIVER CELLS
Mn^{56} / μmole TISSUE NITROGEN
2
1.5
1
0.5
16 %
41 %
15 %
29 %
100%
NUCLEI
MITOCHONDRIA
MICROSOMES
SUPERNATANT
WHOLE LIVER

SLIDE V