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NEUTRON ACTIVATION ANALYSIS:

CLINICAL AND BIOLOGICAL STUDIES OF MANGANESE *

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INTRODUCTION

A strong motivation for metabolic investigations of essential metals lies with the simple fact that these elements are primordial metabolites and building blocks of living matter, a truism largely forgotten today. Among them, the metals of the first transition group display high physical and chemical flexibility: They can concommitantly coordinate natural or artificial ligands; they can regulate electron and proton transport; they can act as catalysts or as cofactors to catalysis.

In spite of Bertrand's early demonstration of the importance of trace metals in biology, definitive studies have been handicapped chiefly by the minute concentration of some of these agents. This fact is illustrated in Figure 1, where metals of proven essentiality are shown as rough estimates of total body concentrations in a 70 kg. man. The ordinate is logarithmic, indicating a smooth, sharp fall of the concentration of these metals. This slide also suggests that studies of the "trace" metals shown in this series require specialized methodology if one is to avoid experimental distortions of biological realities. Isotopic techniques for example, may define the dynamic behavior of these elements *in vivo* or *in vitro*, and magnetic techniques have been useful in determining their state. Needless to say that these approaches are fully fruitful only with adequate quantitation of the metals under study. We shall try to show that neutron activation analysis is a potent method in this context; not only because of its high sensitivity and specificity but particularly its inherent potential to guard the samples against both contamination and loss.

Our interest has been focused on one microconstituent, manganese. This metal is essential to all living things hitherto tested (Cotzias, 1962). In spite of its essentiality, exposure to some of the metal's compounds induces in man an extrapyramidal disease (manganism or chronic manganese poisoning) (Cotzias, 1958) which will be discussed briefly later.

Work at this Center has shown that manganese pursues a specific pathway through the body (Cotzias & Greenough, 1958) which leads largely through the mitochondria (Maynard & Cotzias, 1955 & Hughes & Cotzias, 1961) and to a much smaller extent through a manganoporphyrin of red cells (Borg & Cotzias, 1958 ; Norris & Klein, 1961). The above considerations supplemented numerous biochemical experiences (Cotzias, 1962 ; Cotzias, 1958) which indicate that manganese is a cofactor to several enzymatic reactions and that it can interact with at least one group of organic electron donors to produce free radicals (Cotzias & Borg, 1962 ; Borg & Cotzias 1962).

From both the medical and the biological points of view, it was necessary to quantitate this trace element in small samples of manganese-poor pools (plasma, spinal fluid, bile, etc.) with high precision and accuracy, but without either contamination or loss. With such a method we could attempt to define normal and distorted homeostasis of this element as well as its role under conditions of disease and of therapy. These problems will be defined as the paper proceeds.

NEUTRON ACTIVATION ANALYSIS

The collection of samples, preparation of receptacles and general handling prior to introduction into the reactor are detailed elsewhere (Papavasiliou & Cotzias, 1961).

Two reactors were used in this study: 1) The Brookhaven National Laboratory Graphite Reactor (17-20 megawatts, 1.17×10^{13} neutrons per cm^2 per second, "Cadmium ratio" about 38, temperature 90° C.) 2) The Medical Reactor (3 megawatts, 1.12×10^{13} neutrons per cm^2 per second, "Cadmium ratio" 71.5, temperature 71° C).

Data from these two reactors were concordant. Hence it is planned to eventually use the High Flux Reactor presently under construction at this Laboratory (Kouts, 1961). It is expected that less elaborate chemical separation than that practiced hitherto will suffice in the quantitation of some ensuing short-lived isotopes, including manganese 56.

Since the only stable manganese isotope is Mn^{55}, neutron bombardment yielded the following reaction: Mn^{55} $(n,\gamma)Mn^{56}$, ($\frac{T}{2}$ = 2.58 hours decaying by energetic beta and gamma emission). Production of Mn^{56} during neutron bombardment of biological materials may occur by two additional reactions: 1) Fe^{56} $(n,p)Mn^{56}$; 2) Co^{59} $(n,\alpha)Mn^{56}$. These are fast neutron reactions. Their contribution to the Mn^{56} produced by thermalnneutrons was estimated to be negligible for all tissues other than blood, where iron must have contributed 4-5% of the Mn^{56} (Papavasiliou & Cotzias, 1961).

Following activation, the samples and the standards were digested in the presence of known amounts of manganous sulfate as the carrier. There is a long standing custom in the case of <u>tracers</u> of polyvalent metals, to establish that the tracer is a true tracer of the metal under study. The reverse is necessary in the practice of neutron activation analysis: to demonstrate that the <u>carrier</u> is a true carrier of the element of tissues. In the present work, this was demonstrated as follows: Mn^{54} was injected into a rat which was sacrificed fifty days later. During this time the

isotope must have undergone redox changes and must have become equilibrated with natural manganese pools within the animal. The isotope was then recovered from liver, brain, lung, kidney and muscle by the carrier procedure recommended for neutron activation analysis. This indicated that the carrier was a true carrier of tissue manganese.

Losses of sample were shown to be negligible by processing tagged serum in a manner identical to serum analyzed by neutron activation. Measurements of the recovered carrier were adopted as additional safeguards against loss of sample.

Contamination was possible only during the steps which precede neutron bombardment. Listed below are the major offenders and their controls: 1) Inadequately cleaned distillation flasks: The ensuing distilled H_2O or HNO_3 would contaminate (instead of cleaning) the tubes which were to receive the samples. Monthly cleaning of distillation apparatus was adequate. 2) Metallic instruments including needles. Quartz knives, plastic forceps, plastic "glassware", platinum lumbar puncture needles and pure aluminum venipuncture needles have controlled this error. 3) Dust. 4) It should be stressed that all reagents-grade chemicals hitherto analyzed (with one exception) contained significant amounts of manganese. It is noteworthy that dialysis of hemolysate against 0.005 M Ethylenediaminetetraacetate induced some contamination of the sample with manganese. Heavy contamination was induced into 5 ml aliquots of plasma after dialysis against a bath containing of 1200 ml of 0.9% NaCl. The salt had been obtained from an optical crystal which had been reported earlier as not containing demonstrable manganese in samples weighing about 1 mgm. This was the one reagent which was thought to be uncontaminated (Papavasiliou & Cotzias, 1961).

This element cannot be the only contaminant merely because it was the only one tested. Neither can it be assumed that the increase of manganese concentration is the only biochemical consequence of contamination. Onethe contrary, one cannot exclude enzymological and other consequences. These remarks do not apply only to extrinsic contamination. Indeed the high concentration gradients which are characteristic of many adjacent compartments of the body make the problem of intrinsic contamination germane: For example, blood obtained by pricking and squeezing the finger contained higher and more variable concentrations of manganese than did samples obtained by venipuncture. This type of contamination might be even more important when one samples micro-compartments such as intracellular inclusions or individual macromoles. Unlike extrinsic contamination, the intrinsic type cannot always be detected by means of accounting for the concentrations in fractions relative to the concentration in the original sample.

Chemical separation consisted of oxidation of the digest to permanganate and precipitation of the permanganate with tetraphenylarsonium chloride. The latter was facilitated by filtering on a millipore disc, which was inserted thereafter into a planchette and counted on an assembly containing a multichannel analyzer. This permitted computation of the entire Mn^{56} photo peak at .845 mev.

The effect of this chemical separation on the determination of Mn^{55} of plasma and blood is shown in Figure 2. Figure 3 shows the correlation of Mn^{55} added to serum with that determined analytically. Eleven individual samples from the same serum showed a mean and standerd deviation of 2.61 ± 0.09 μg of Mn^{55} per liter. (Papavasiliou & Cotzias, 1961).

CLINICAL AND BIOLOGICAL STUDIES

The present technique appeared applicable to the detection of macromolecular complexes of manganese in plasma and in blood. This study was motivated by two considerations. First, various natural macromolecular ligands of other essential metals (zinc, copper, iron) have been studied in detail while manganese containing ones have not been identified. Their existance has been suggested by studies with artificial isotopes (Cotzias, 1962 ; Cotzias, 1958 ; Borg & Cotzias, 1958 ; Norris & Klein ,1961 ; Cotzias & Bertinchamps, 1960) and it was necessary to confirm these isotopic studies. A second consideration was based on the fact that in most nutritional, physiological, enzymological and other experimentation, an inorganic form of manganese has been tested (Underwood, 1962), which might not have duplicated the natural complexes of the metal and might have induced the formation of artificial ones in addition. Since blood, plasma and serum are instrumental in the transport of this element, they offered biological interest to the present study (Cotzias,& Papavasiliou, 1962). Spinal fluid, which contains minimal concentrations of protein might be a source of a non-protein-bound form of this element, provided that it contained manganese. Furthermore, the presence of steady concentrations of this metal in these various pools might suggest the presence of homeostatic controls and might encourage the study of homeostasis.

In view of our experience with contamination, it was necessary to avoid the use of reagents unless such reagents were proven to be suitable. Therefore, instead of reagents, a high gravitational field was used in order to separate this metal into macromolecular (sedimenting) and micromolecular (supernatant) fractions. Centrifugation of spinal fluid at $11 \times 10^4 \times G$ for

forty-eight hours yielded a supernatant fraction which was protein free by nephelometry (Looney & Walsh, 1939). This fraction contained 93-94% of the metal. Similar but more prolonged centrifugal fractionation of human plasma yielded variable concentrations of Mn^{55} in the protein-free supernatant fraction which ranged from 4 to 25% of the plasma manganese on repeated duplicate analysis. This was concordant with unpublished isotopic studies which indicate that while the total manganese in plasma might be constant, its state of binding and its partition among plasma proteins may be variable indeed.

Similar experimentation with hemolysates showed about 90-96% of red cell manganese to be sedimentable with the proteins.

In earlier isotopic experiments, it was found that the injected $Mn^{54}Cl_2$ becomes only loosely bound to plasma proteins (Cotzias & Bertinchamps, 1960) while it becomes firmly incorporated into the hemoglobin heme of human and rabbit red cells (Borg & Cotzias, 1958). If the artificial isotope had truly traced the natural species of this element, one should show markedly different states of binding of plasma-bound versus hemoglobin-bound Mn^{55}. This proved to be the case when dissociation by proton competition was employed. Acidification of plasma with distilled hydrochloric acid (final pH 4.3 - 4.5) followed by high-speed centrifugation yielded protein-free supernatant fractions containing 85-100% of the total manganese. In striking contrast, similar treatment of hemolysates yielded only 17-24% of the total metal in the supernatant, protein-free fraction. These tests indicated that the receptor sites for manganese are different in plasma than they are in red cells, a fact which is concordant with the isotopic experience (Borg & Cotzias, 1958 ; Norris & Klein, 1961).

These results pertaining to partition of this element among centrifugal fractions, were expressed as percents of a total which was not defined above. Actual concentrations of manganese have been determined on human samples at this Center. Following are the means (μg Mn/Liter) and stand deviations: 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

These analyses were performed on individual hospital patients who were not under standardized conditions with regard to diet, treatment, medication or the timing thereof relative to sampling. Even so, the variance in these series is small indeed. Among the plasma values the variansis even less than that presented earlier with regard to the clearance of intravenously injected $Mn^{56}SO_4$ (Borg & Cotzias, 1958).

The relatively small scatter of these data strengthened the notion gained on the basis of isotopic experiments that manganese, like zinc (Cotzias, Borg & Selleck, 1961 ; Cotzias, Borg, Selleck, 1962) is under homeostatic control within the body. Nonetheless, in one patient, feeding of massive amounts of a manganese salt (2.0 gm $MnSO_4$ daily) increased the red cell and spinal fluid concentration without causing much increase of the plasma level. Upon discontinuation of the $MnSO_4$, these values regressed. The rate of loss of intravenously injected $Mn^{54}Cl_4$ from the whole body and from the liver of this patient were tested before and during this massive manganese intake. A marked acceleration of this loss was observed with both measurements as a consequence of the administration of this salt. The sum of these experiments indicates that while manganese could become excreted at an accelerated rate, this acceleration was not always sufficient to prevent its accumulation in at least two of the components of the body. Hence, the postulated homeostatic controls may become saturated.

In earlier isotopic experiments on mice (Hughes & Cotzias, 1961) it was found that minerocorticoid hormones (cortisone, prednisone, prednisoline) markedly affected the metabolism of manganese. We became intrigued by the possibility that the metabolism of this element might be effected also inppatients who need to be treated with such hormones. In an interdisciplinary meeting such as this, it might be necessary to explain some of the medical interest in these hormones: From the therapeutic point of view these agents are often lifesaving in the manggement of collagen diseases (rheumatoid arthritis, lupus, etc.) asthma, anaphylaxis, infectious hepatitis, leukemia and other life-threatening conditions. From the physiological point of view, these agents are of interest chiefly because of their homeostatic role in the body. This is particularly evident in the response to stress which might follow surgery, cold, trauma, infection, anaphylaxis, etc.

Six unpublished cases of rheumatoid arthritis were hitherto treated at this Center with high doses of prednisone, a cortisone analogue. Figure 4 shows the behavior of intravenously injected $Mn^{54}Cl_2$ in one such case. In response to the treatment the radioactivity was eliminated at an accelerated pace from the whole body and from the liver, while at mid-thigh the normal rate of loss was decelerated. This indicated that the hormone had caused both a loss of this element out of the body and a redistribution from one organ to another. These effects could manifest themselves only if the bloodstream had been transporting manganese at an increased flux during the treatment as compared to either before or after the drug was administered. If the change in flux had been marked, one might have been able to detect a concordant rise and subsequent fall of the actual concentration of this metal in the plasma. Figure 5 shows this to be indeed the case. The total

body radioactivity curve was plotted again in order to show the time at which the natural manganese was determined in plasma relative to the accelerated excretion of the radioactive species of this element.

These studies are still inpprogress. Therefore it is too early to decide whether the changes in manganese metabolism induced by these hormones pertain to their therapeutic effect or whether they indicate (as does the potassium level) that special precautions are necessary to combat possible manganese deficiency during the treatment of such cases. Regardless as to what explanation will prevail, neutron activation analysis of plasma for manganese appears to emerge as a possible clinical necessity.

A consequence of surgical stress (which as yet cannot be ascribed to these hormones with certainty) was observed while studying the biliary excretion of manganese (Bertinchamps & Cotzias, 1958). An example of these experiments is shown on Figure 6. Bile was collected from a rat shortly after laparotomy, by means of a catheter located in the common bile duct. Carrier-free $Mn^{54}Cl_2$ had been injected intravenously at the outset. While the specific activity of the isotope fell, a marked rise appeared in both Mn^{55} and Mn^{54} within the bile. In other experiments, this rise had been followed by a return to basal levels (Papavasiliou & Cotzias, 1961). Nonstressed animals excrete Mn^{54} asymptotically into their bile, in a manner similar to the excretion of injected radioisotopes by the kidney.

The concommitant consideration of both biliary and renal functions is particularly meaningful in the case of manganese metabolism (Cotzias & Papavasiliou, unpublished). The kidney has been studied extensively with reference to its <u>excreting</u> as well as with regard to its <u>regulating</u> the flux

of many substances, in what Claude Bernard called the "milieu interieur". Indeed, it is considered as the main homeostatic end organ in mammals.

The kidney is fully developed only in a relative minority of animal species, namely those high on the evolutionary scale (Arey, 1948). In animals which have not yet developed kidneys, the primordial homeostatic organ is the ancient hepatopancreas, which has evolved into the mammalian liver and pancreas. In mammals, manganese is excreted both into the bile (Greenberg,& Copp & Cuthbertson, 1943) and into the pancreatic juice (Burnett, Bigelow, Kimball & Shapard, 1952) but certainly not into the urine (Cotzias & Greenough, 1958); Cotzias & Papavasiliou, unpublished). Hence, the hepatopancreatic successors seem to have maintained their adequacy with regard to the excretion of manganese in mammals. If the same organs are adequate also in the control of the flux of this element, they must have remained adequate for the homeostasis of at least this one primordial metabolite, independently of the continuing evolution of the species.

In this regard, three pertinent questions seemed to be readily amenable to experimental testing: 1) Does obstruction of the bile duct decelerate the excretion of manganese? 2) Does such an obstruction affect the animals' capacity to respond with accelerated excretion to a metabolic burden? 3) Does it result in abnormal concentrations of the substance under study?

Answers to the first two questions were sought by means of isotopic techniques, while the third was answered by both neutron activation analysis and the use of Mn^{54}. It must be noted that evidence of the effects of surgical stress was found again in the present experiments (Papavasiliou & Cotzias, unpublished) in spite of the fact that this time, the laparatomies were not performed in order to drain the bile, but instead, in order to obstruct its flow.

The excretion of injected Mn^{54} was studied in rats by means of total body counting. Complete, apparently permanent abolition of the isotope's excretion was achieved only by obstructing the anus. Biliary obstruction always resulted in some degree of deceleration of the isotope's excretion, which differed when other routes were used for the injection of the isotope. When the isotope was injected into the portal vein, the most marked difference between obstructed and control animals was seen, indicating that the biliary manganese represents primarily hepatic manganese (Cotzias & Papavasiliou, (unpublished).

A striking consequence of biliary obstruction was seen in animals after they were given a dietary supplement of $Mn^{55}SO_4$ in their milk, which is a manganese deficient food. (Cotzias & Greenough, 1958); Underwood, 1962), The rate of excretion of manganese 54 is slow in the case of milk-fed animals, and biliary obstruction decelerated it even more. When 2.2 γ of Mn^{55} were added per ml to the milk of the obstructed animals, no acceleration in the excretion of Mn^{54} was evident. However, in the case of control animals, feeding of one half of that amount of manganese supplement resulted in a marked acceleration of the isotope's excretion. The sum of these experiments indicated that while more than one gastrointestinal tributaries serve to excrete manganese, it is the liver which responds to ordinary metabolic demands by regulating the metal's flux. Experiments with graded, massive, parenterally administered doses of $MnSO_4$ indicated that the bile-obstructed animals also can be forced to respond to a metabolic burden if this burden is large enough. Nonetheless, even here biliary obstruction had curtailed both the magnitude and the rapidity of the response.

Since biliary obstruction causes a diminution of an animal's capacity to excrete as well as to regulate the flux of manganese, one would expect an accumulation of this element proximally to the obstruction. Indeed such an accumulation was shown to occur by testing for both the radioactive and the natural species of this metal. Abnormally high concentrations were encountered primarily in the liver but not in other tissues tested. This might have been due merely to the fact that these experiments were continued for a maximum of nine days, during which time equilibration of the hepatic pool with the other pools of the body might not had established itself.

These experiments must be pertinent relative to other forms of jaundice, since jaundice is obviously a grave derrangement of homeostasis and one form of jaundice (the experimental obstructive variety) was shown to affect the homeostasis of manganese.

Other derrangements of this element's homeostatic control were alluded to earlier in this paper. One intriguing such derrangement is chronic manganese poisoning. Investigations of this disease are of pertinence to the study of extrapyramidal diseases in general (Cotzias, 1958). A tenuous link between manganese and some drugs which have both induced and alleviated human extrapyramidal diseases has been discussed in some earlier publications from this Center (Cotzias & Borg, 1962 ;gBorg & Cotzias, 1962); Cotzias, Borg, Bertinchamps, Hughes & Papavasiliou, 1961). These drugs are derivatives of phenothiazine. One such drug is the common tranquilizer thorazine (Largactyl).

Aqueous solutions of these agents were permitted to react with manganic oxide-hydroxide (Mn^{+++}). Highly colored metastable compounds were observed which were identified as semiquinone free radicals of these drugs by means of electron spin resonance spectroscopy (Borg & Cotzias, 1962).

These free radicals were not produced when some pharmacologically inactive compounds were tested, but only with therapeutically useful forms of these drugs. In spite of this parallelism it was necessary to test also some biological system for evidence of an interaction between manganese and phenothiazine drugs leading to the formation of free radicals. For reasons which are not pertinent to this discussion (Cotzias & Papavasiliou, unpublished), such a test was considered as difficult for the present. Hence an alternate approach became necessary, namely to seek for some biological structure known to contain semiquinone free radicals and to determine whether it contains manganese. The concommitant presence of free radicals and manganese in one and the same site would tend to encourage further correlative studies.

Some recent work indicates that melanin contains semiquinone free radicals (Mason, Ingram & Allen, 1960 ; Longuet-Higgins, 1960). Additional work has shown that dark human hair tends to contain a higher concentration of free radicals than does light-colored hair (Kerkut, Edwards & Munday, 1962). Furthermore, melanin granules isolated from the bovine choroid were reported to exhibit a higher electron spin resonance signal when exposed to light than in the dark (Cope, Sever & Polis, 1962). Hence melanin granules emerged as suitable sites for testing possible correlations between manganese and free radicals. This had to be achieved again without sacrificing the inherent guarantee of neutron activation analysis against contamination, namely without the use of reagents. This meant that one could not isolate the melanin granules. Therefore, controlled experiments were conducted first by analyzing hair from six individuals who had both dark and white hair on their heads and comparing the two. The concentration of manganese in

hair varied markedly from one person to the next. Nonetheless, for any given person, the element's concentration was higher by at least a factor of two in the dark as compared to the control specimen. Since only two shades of darkness could be discerned accurately when separating human hair, animals (dogs and cows) were sought with more than two colors. The results confirmed those obtained with human hair. Still, these differences in manganese concentration among the pigmented and the control specimens might have reflected some local factor rather than only the actual concentration of pigment, in view of the fact that each hair grows from a different follicle, and the constitution of hair might reflect local conditions of the skin. In birds, however, a single follicle often produces a feather with multicolored barbs. Analyses of such barbs confirmed the previous experiments.

The sum of these tests indicated that the pigments studied constitute a site of high concentration of manganese. One might argue however, that this was demonstrated only for non-viable tissues. Therefore, living tissues were sought which might permit similarly controlled experimentation. The bovine conjunctiva has dark and white portions. The dark portions are adjacent to the animal's cornea. Hence samples of dark conjunctiva, white conjunctiva and cornea were activated and their Mn^{55} concentration was tested. These experiments indicated that melanin granules in general might constitute a site of high accumulation of this metal.

By the same token, there emerged a possible discrepancy between the above statement and our earlier claims, that it is the mitochondrion which accumulates preferentially two artificial species of manganese, namely Mn^{56} (Maynard & Cotzias, 1955) and Mn^{54} (Hughes & Cotzias, 1961)./ A recent

paper (Dorner & Reich, 1961) indicates however that melanin granules and mitochondria might have certain similarities of functions in spite of their dissimilar constitution and structure. Preliminary tests at this Laboratory on melanin granules from Amphiuma liver (Van Woert & Cotzias, unpublished) indicate that these bodies contain high concentrations of Mn^{55}. Furthermore they also accumulate Mn^{54} to about the same level as do the animal's mitochondria. These pigmented structures also seem to contain a high concentration of the enzyme monoamine oxidase (Cotzias & Dole, 1951) according to experiments still in progress. It should be noted that radiomanganese had been proposed as a means of studying mitochondrial function in the intact man (Borg & Cotzias, 1958) and that monoamine oxidase had been localized in the mitochondria of rat liver (Cotzias & Dole, 1951) & Cotzias, & Serlin & Greenough, 1954). Hence it appears that the melanin granules of Amphiuma liver display two features in common with mitochondria and will be studied further.

CONCLUSIONS

Various arguments were elaborated in this paper, during the evolution of which there were mentioned at one point or another, neutron activation analysis; manganese, and some of its radioisotopes; phenothiazine drugs; free radicals; extrapyramidal diseases; homeostatic regulation and aberrations thereof. The paper was concluded by discussing melanin granules. Do all these diverse entries lend themselves to some unification of additional usefulness besides the elaboration of the arguments already presented? They do.

The higher mammals and man are sensitive to spontaneous experimental extrapyramidal disease while lower animals appear to be refractory. The higher mammals have pigment granules in the substantia nigra of their brain, and the lower ones do not. In man, some extrapyramidal diseases are characterized at autopsy by a loss of this pigment. The pigment granules examined to date contain various metals (including manganese) as well as free radicals. Therefore a high probability emerges that concentrated investigations of these pigment granules might serve to unify studies of various extrapyramidal diseases of variable induction and etiology.

LEGENDS

Figure 1 — Total body content of metals with proven structural or functional roles, Computed from Altman, P.L. (Editor) Blood and Other Body Fluids, Federation of American Societies for Experimental Biology (1961); Spenser, William S. (Editor) Handbook of Biological Data W.B. Saunders Co. Phila.(1956); and Tipton, I.H., Cook, M.J., Health Physics (1963) 9: 103. The bulk metals are in white, the trace metals in black. Highly sequestered iron of myoglobin, hemoglobin and bone is shown in white. Trace iron is in black.

Figure 2 — The gamma ray spectra of 100 µl of blood and serum are shown at the top. The corresponding purified radioactivities are at the bottom. (From Papavasiliou, P.S. and Cotzias, G.C. (1960) J. Biological Chem. 236: 2365. Reproduced by permission of the Editors.

Figure 3 — Solid circles correspond to serum samples to which manganese was added. The open circle depicts the manganese concentration of that serum. (From Papavasiliou, P.S. & Cotzias, G.C. (1961) J. Biological Chem. 236: 2365.

Figure 4 — Change of the Mn^{54} concentration in the whole body, the liver and the midthigh of a woman who received prednisone.

Figure 5 — Same as Figure 4 but with plasma manganese concentration plotted.

Figure 6 — Change in the concentration of Mn^{54}, Mn^{55} and specific activity in the bile of a rat under surgical stress.

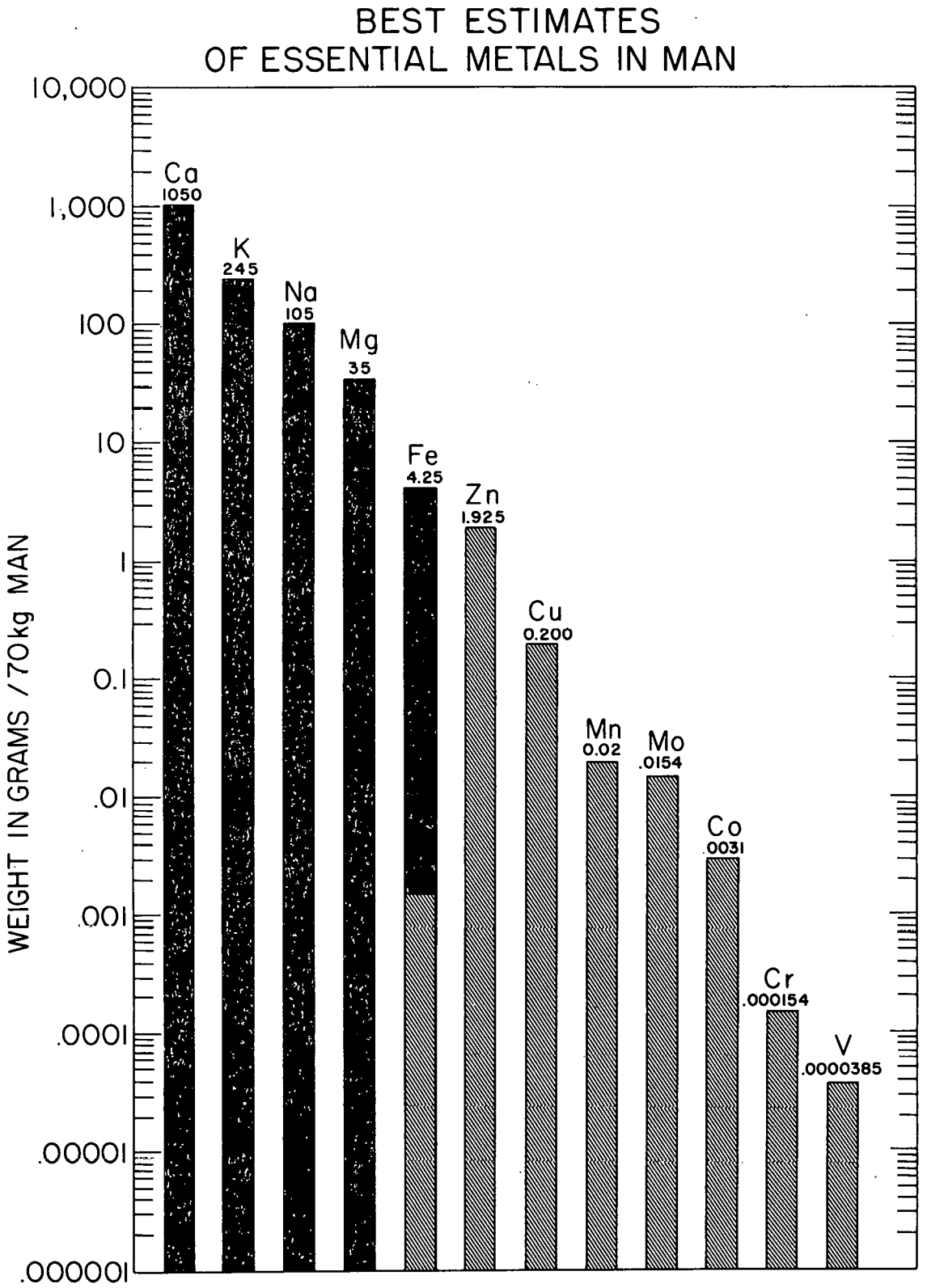
BEST ESTIMATES
OF ESSENTIAL METALS IN MAN
WEIGHT IN GRAMS / 70kg MAN
10,000
1,000
100
10
1
0.1
.01
.001
.0001
.00001
.000001
Ca
1050
K
245
Na
105
Mg
35
Fe
4.25
Zn
1.925
Cu
0.200
Mn
0.02
Mo
.0154
Co
.0031
Cr
.000154
V
.0000385

FIGURE 1

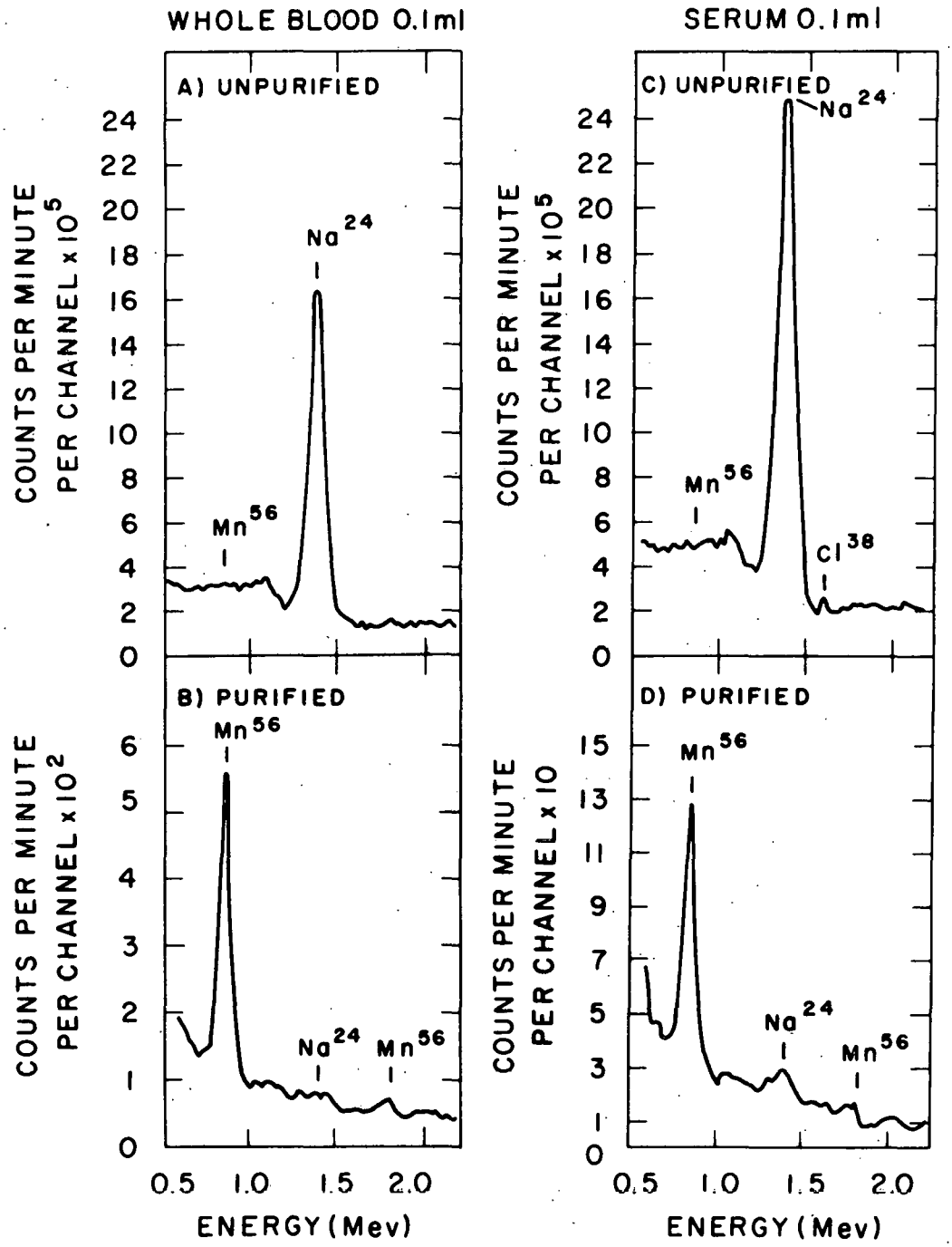
WHOLE BLOOD 0.1ml
SERUM 0.1ml
A) UNPURIFIED
B) PURIFIED
C) UNPURIFIED
D) PURIFIED
Na24
Mn56
Cl38
COUNTS PER MINUTE PER CHANNEL x 10^5
COUNTS PER MINUTE PER CHANNEL x 10^2
COUNTS PER MINUTE PER CHANNEL x 10
ENERGY (Mev)
0.5
1.0
1.5
2.0

FIGURE 2

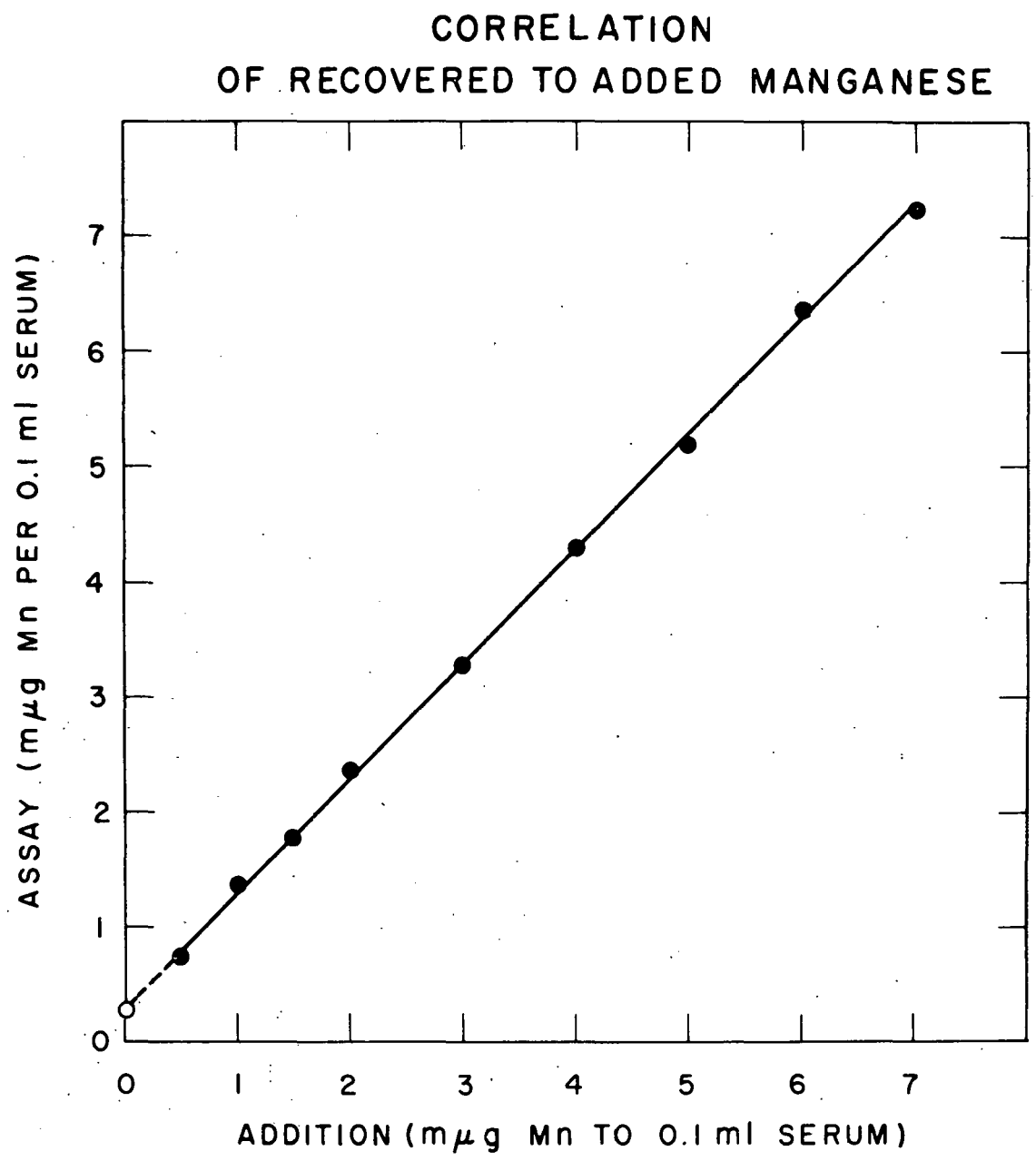
CORRELATION
OF RECOVERED TO ADDED MANGANESE
ASSAY (mμg Mn PER 0.1 ml SERUM)
ADDITION (mμg Mn TO 0.1 ml SERUM)
0
1
2
3
4
5
6
7

FIGURE 3

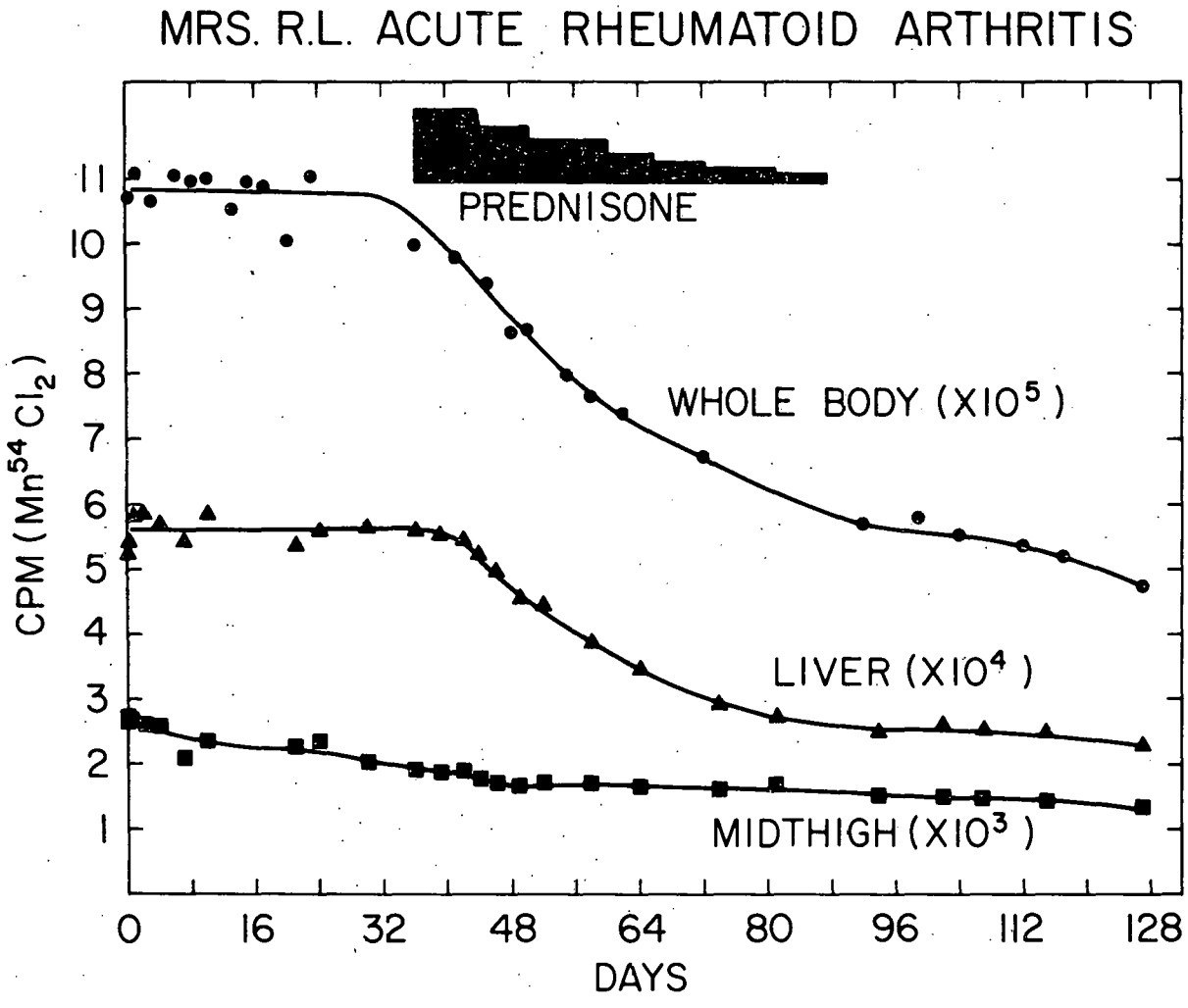
MRS. R.L. ACUTE RHEUMATOID ARTHRITIS
PREDNISONE
WHOLE BODY (X10⁵)
LIVER (X10⁴)
MIDTHIGH (X10³)
CPM (Mn⁵⁴Cl₂)
1
2
3
4
5
6
7
8
9
10
11
0
16
32
48
64
80
96
112
128
DAYS

FIGURE 4

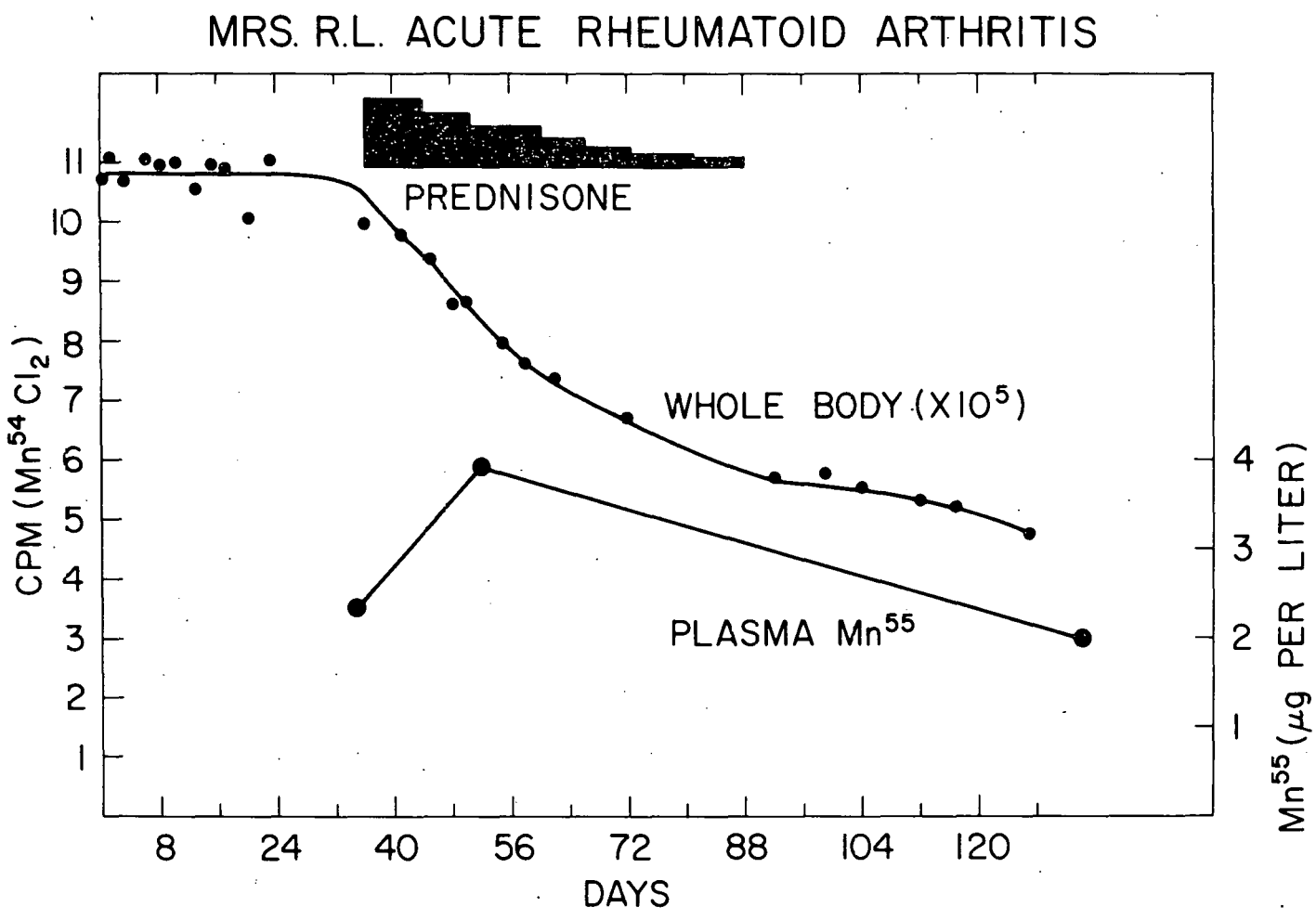
MRS. R.L. ACUTE RHEUMATOID ARTHRITIS
PREDNISONE
WHOLE BODY (X10^5)
PLASMA Mn^{55}
CPM (Mn^{54}Cl$_2$)
Mn^{55} (μg PER LITER)
DAYS
1
2
3
4
5
6
7
8
9
10
11
8
24
40
56
72
88
104
120

FIGURE 5

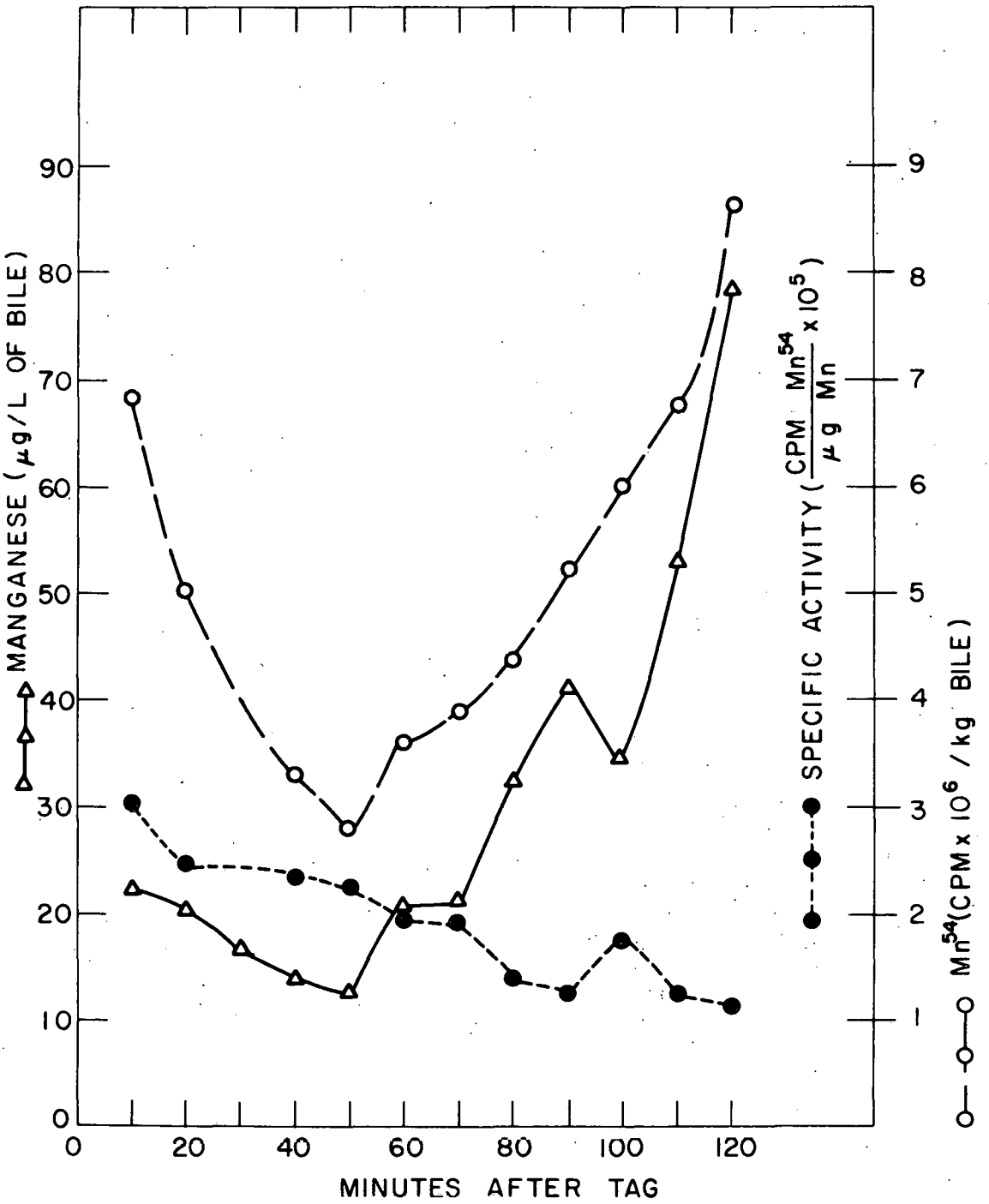
MANGANESE (μg/L OF BILE)
SPECIFIC ACTIVITY (CPM Mn54 / μg Mn x 10^5)
Mn54 (CPM x 10^6 / kg BILE)
MINUTES AFTER TAG
0
10
20
30
40
50
60
70
80
90
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
120

FIGURE 6

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