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TRANSPORT, HOMEOSTASIS AND SPECIFICITY IN TRACE METAL METABOLISM * +

George C. Cotzias

Medical Research Center
Brookhaven National Laboratory
Upton, Long Island, N.Y., U.S.A.

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I am very grateful for having been invited here today. It so happens that many of the physiological observations on which I will touch have been consummated by my associates (see footnote 1) and myself, in part at least, by means of techniques borrowed from the Nutritionists. This gives me great pleasure because it indicates how kindred our respective disciplines really are. Indeed, one of the attractions of working in this field is its interdisciplinary nature.

I will tell you today about some continuing studies on the physiology of trace elements, notably of manganese. I intend to spend some of your time discussing various aspects of normal and perturbed homeostasis of these elements.

A working definition of homeostasis is the following: the sum total of the processes which maintain a steady level of various substances or functions within a living body. Our own concern will be with the homeostasis of trace metals. I will begin with a slide (Fig. 1) which shows the total body concentration of various metals, including "trace" ones. Only metallic constituents which play a role in the maintenance of the body's structure and function are shown. The essential trace metals among them are represented by black bars. Iron is both black and white. Indeed, if one excludes the fixed, sequestered iron of myoglobin and of hemoglobin, one is left with a trace fraction of iron about which I will be talking later on. Note that the scale is logarithmic, indicating that the concentrations of essential metals in the body fall off sharply and smoothly.

The essential trace metals, namely those which are instrumental in the maintenance of structural and functional integrity of living things seem to be ubiquitous in all living organisms. It is readily comprehensible that

diminution of their concentration in the body should cause reproducible damage, since this is actually how Nutritionists have discovered essentiality. What is intriguing however, is that too much "trace" may cause predictable disease also. This shows the impottance of the rigorous controls of "homeostasis" even though only tiny amounts of essential material may be at stake. Furthermore, the mechanisms by which a derangement of homeostasis can occur are diverse and therefore most intriguing. A relatively simple mechanism is represented in manganese poisoning where inhalation of mangenic ore dusts leads to random disease among miners. A more complex mechanism operates in Wilson's disease, a familial, genetically transmitted illness. Here, normal dietary copper is concentrated within the body, leading eventually to copper poisoning. Both Wilson's disease and manganese poisoning strike at the central nervous system. Since other spontaneous diseases are also ascribed to excesses of trace elements; it is fair to claim that many degenerative diseases, presently of unknown origin will prove to belong in this category. For the moment, however, let us stay with Wilson's disease and manganese poisoning. These illnesses present us with some very intriguing similarities between each other and with Parkinson's disease. Indeed all of them are grouped together by Neurologists under the heading of "extrapyramidal diseases". Let's look at a short movie illustrating these similarities.

In this movie you will see two North American patients from our own Hospital at Brookhaven National Laboratory, being compared to two Chilean <u>mineros</u>, whom we photographed in La Serena, Chile last fall.

The first, a North American woman suffers from Wilson's disease. Look at her gait, her expression, the bizarre movements of her hands. Her

Chilean counterpart has one of the three forms which manganese poisoning can assume (see footnote 7). Yet, look at his gait, his facial expression and the distorted movements of his hands. Isn't he similar to the first case?

The second North American has a disease of unknown origin, Parkinson's disease. Look at his posture, his mask-like face, and the poor movement of his arms. Now, compare him to the second Chilean who suffers from still another form of manganese poisoning; look how similar he is to the American who has Parkinsonism.

As you have seen, there are some general as well as some specific similarities among these patients. It is a complete mystery at the moment why any clinical similarity whatsoever should exist between diseases of totally dissimilar etiology such as these. Complete mysteries sometimes make intriguing problems for research. I hope to show you at the end of this talk that we are ready to attack this problem.

At the outset of this work, we were particularly struck by two facts which I have not told you yet. The facts are significant because they constitute a most powerful argument relative to the importance of essential trace elements in the phenomenon of life. First, the essential trace metals were present at the inception of life and have remained essential to all living things hitherto studied. Second, these metals are transported -- in the mammalian at least -- by protein molecules. Examples of such proteins are ceruloplasmin, instrumental in the transport of copper: transferrin, in the transport of iron; and other proteins. Now, let's take transferrin for example: It has a molecular weight of about 100,000 but it carries normally only about one atom of iron, (atomic weight about 56) per molecule. This

means that the body constantly activates an enormously complex mechanism to synthesize about 2000 units weight of carrier protein which transports only one unit weight of trace metal. If one were to ascribe evolutionary meaning to these two facts, it would make more sense to attribute primary evolutionary function to the simple but primordial trace elements than to the complex newcomers, the macromolecules.

These are the considerations which led us to study transport mechanisms in the initial phases of our work. The initial experiment was intriguing in its simplicity. We injected $Mn^{56}Cl_2$ (half life: 2.5 hours) (1) or $Mn^{54}Cl_2$ (half life 314 days) (2) intravenously into animals. To our surprise, the element disappeared most rapidly from the bloodstream. The next obvious question was: Where is it heading so fast? This was determined by means of dissection of animals that had been injected intravenously with radiomanganese. We ranked the organs of the animals on the basis of their relative enrichment with isotope. This gave us catalogues of organs the ranking of which remained identical from mouse, to rat, to rabbit and, as far as we can tell from surface scanning, to man. One such catalogue is shown on the next slide, (Fig. 2). This constant ranking was interesting because it seemed to indicate something significant about mammals in general rather than about radiomanganese in particular.

The highest concentrations of isotope per gram of tissue occurred in liver and the pancreas, while the lowest concentration occurred in skeletal muscle. Spleen and lymphnodes were rather poor, indicating that scavenging of the isotope by the reticuloendothelial system, was not the cause for this distribution. It occurred to us that organs rich in manganese are also rich in mitochondria, which as you know, contain most of the respiratory enzymes.

including those of oxidative phosphorylation. Hence it became important to see whether there was a correlation between manganese concentration and mitochondria.

This question was answered by injecting animals with one of the manganous radioisotopes and fractionating the cells by centrifugal fractionation. This yielded four fractions: mitochondria, microsomes, a heavy (or nuclear) fraction and the soluble materials of the cells. The results were what we had expected: About 40% of the radioactivity was associated with the mitochondria isolated from rat liver and these bodies contained about twice the radioactivity which was found in the intact liver. The next slide (Fig. 3) illustrates these points. The bars show the relative specific activity of these fractions, namely radioactivity per unit nitrogen. The numbers on top of the bars indicate the percent of the total radioactivity of the liver located in each of these fractions. Due to the several washings inherent in this procedure, the values assigned to the mitochondria constitute minimal estimates, while those of the soluble fraction were overrated.

In our clinical work, this experiment started us wondering whether we had not invented a clinical method for measuring mitochondrial function. This was imagined as something like the iodine uptake tests for the thyroid, but instead of testing the function of an organ, we would be testing the behavior of intracellular organelles. We investigated this possibility in 19 patients and the results are shown on the next slide (Fig. 4). Look how fast this radioisotope disappears from the bloodstream after intravenous injection. In spite of this rapidity, the range of these data is narrow indeed. If one now resolves this multicomponent clearance curve into its constituent parts, one gets the three components shown on the next slide (Fig. 5). Among these,

we have proposed the middle one ($\frac{T}{2}$ = 2.65 min.) for the estimation of the relative size of the mitochondrial pool and for the measurement of the manganese turnover therein (3). This was done because this component fits the tissue uptake data best. This proposal is probably correct because it is this component which changes in thyrotoxicosis (4), a mitochondrial disease par excellence, in which oxidative phosphorylation is uncoupled. Furthermore, we seem to be confirmed by Baxter and Smith in their work concerning liver injury induced by carbon tetrachloride in rats (5). These investigators found a very marked diminution of Mn^{54} uptake by the liver while another transition metal, Zn^{65} did not reflect this primarily mitochondrial injury. Hence it is evident that Mn^{54} or Mn^{56} are useful clinical tools in the detection of mitochondrial damage either throughout the body or in an individual organ such as the liver.

Since observing radiomanganese <u>disappear</u> was a useful task, one might wonder whether there was not something to be learned by watching this isotope <u>reappear</u> into the blood stream, provided, of course, that it was inclined to do so. Indeed, the isotope reappeared after 7 - 8 days, as you can see on the next slide (Fig. 6). Here you see a log-log plot of the Mn^{54} radioactivity against time (6). This isotope disappeared as fast as Mn^{56} had on the earlier slide, although the log-log scale distorts these data, so that they look different from those I showed you earlier.

It is evident that the wave of red cellbound radioactivity lasts as long as a red cell does, namely about 128 days. Hence this radioactivity can measure the life span of a single generation of red cells. This cannot be done by tagging red cells in a test tube, because one tags more than one generation. Furthermore, the earlier methods for tagging red cells in the living human with

Fe^{59} or labelled aminoacids, also tags more than one generation of red cells: these labels are reutilized by the bone marrow while radiomanganese is not.

Now look at the next slide (Fig. 7). Separation of the plasma (in which this isotope was located at the time of its injection) from the red cells showed the plasma to be free from radioactivity, while red cells contained all of the Mn^{54} found in blood. Separation of the red cells into hemolysate (or soluble) and stroma (or insoluble) fractions, showed that the isotope was located primarily in the hemolysate. The hemoglobin was separated into heme and globin. When this was done most of the Mn^{54} was recovered with the heme. It seemed firmly bound to heme, since it was nondialysable, non-exchangeable and not available for binding by Ethylene-diamine tetracetic acid. These and similar experiments indicated that we had found a natural manganoporphyrin in human and rabbit red cells. Dr. Ray Klein kindly confirmed these observations in birds which have different heme synthesizing mechanisms from mammals (7).

The incorporation of manganese in heme awakened an intriguing question: Since iron is the bulk element located in the center of the pyrrole ring of hemoglobin, isn't it possible that manganese might slip into its place, more or less by accident, so to speak? Manganese is element 25, iron is element 26, so they might be confused with each other by the body. Furthermore, many polyvalent metals have been found to be interchangeable with each other in test tube systems. Furthermore, manganese is considered in some circles to be completely interchangeable with magnesium, which is not even a transition metal like manganese, but an alkaline earth (8). Therefore, it became critical to test whether one given metal is readily confused for any other metal by the intact body as is the case in isolated system.[8]

For this we had to devise a new set of criteria based on chasing an injected radioisotope with nonradioactive metals either out of the body or from one part of the body to another. First it was necessary to check whether an injected isotope will be eliminated at abnormal rates if the host animal is given some nonradioactive metal in the form of a metabolic load. If the rate of loss to the outside of the body remained unaffected, would one perhaps find a distorted internal partition of the radioisotope among the various organs or intracellular organelles?

To implement these notions we used a primordial form of what became known later as the total body counter (9). The first such counter is shown in the next slide (Fig. 8), although the instrument currently in use is a well-type scintillation counter. The total body radioactivity is measured right after the injection of an isotope and the subsequent measurements are expressed as percents of the first count. If one now plots the results on semi-logarithmic paper as a function of time, one gets curves like those shown in the next slide (Fig. 9).

Now we are ready to distort these curves by giving these animals various nonradioactive metal salts. In the next slide (Fig. 10) you see the effects of three such elements, namely element 24 (chromium); 25 (manganese) and 26 (iron) in animals that had been injected with manganese 54. It is obvious that manganese does push radiomanganese out of the body. This can happen only if the total amount of manganese in the body is kept constant by some control mechanism. However, for the moment, it is sufficient to stress that radiomanganese is mobile, its mobility can be increased by administration of nonradioactive manganese, but not by other metal salts, including magnesium. We then checked on the internal distribution of manganese 54. We found it

sensitive to stable manganese loads and insensitive to loads with natural metals other than manganese itself. I say natural metals because I strongly suspect that the man-made technetium might well emerge as an experimentally valuable antimetabolite for manganese. With that one exception, these results can mean only one thing: manganese travels alone for most of its pathway through the body. Otherwise, metals of higher abundance, of higher affinity for natural receptors or both, would have replaced this element. As a matter of fact, now that we have gotten a lot of mileage out of this experiment, I will let you in on a secret: It was a totally unnecessary experiment. If manganese could be washed out of tissues by nonmanganese metals, it would probably have been washed out of the bodies of animals many generations ago.

The next obvious question was: Why should the specificity of manganese be radically different in the intact animal as opposed to isolated systems? Among the many ways in which we could seek for an answer, we chose the one which would best serve us in our work as physicians. The intact bloodstream is readily sampled by physicians. Moreover the circulation constitutes also a marked difference between a living animal and an isolated biological preparation. Hence we searched in human plasma for one or more macromolecules that might be carrying this element. If one were present, we planned to see whether it could tell manganese from, say, magnesium. The next slide (Fig. 11) shows two of three such carriers. All three of them seem to prefer manganese to magnesium over a very wide range of concentrations of the latter. The β_1 globulin in these tests displays a much higher capacity for this element than the albumin fraction does. An alpha globulin (not shown here) behaves like albumin. There is a fourth species of manganese which makes up the balance of the total element in plasma, namely that not bound to protein. With electrophoresis, this "free" species is estimated at 40 - 60% of the total

radiomanganese, depending upon the condition of the donor. By ultrafiltration, equilibrium dialysis and sedimentation, "free" radiomanganese amounts consistently to only 4% of the total. The latter value holds also for the stable, natural Mn^{55} (10) which I will discuss later. Hence we cannot ascribe accurate carrying capacities for manganese to the electrophoretic fractions at the moment, because the "free" species was dissociated by the current from one or all three of these macromolecular ligands. With regard to the valence state of the element in these fractions however, we can say that the β_1 globulin seems to prefer a valence of manganese higher than two and lower than four, therefore probably manganic valence III. Albumin on the other hand, prefers valence II. These results indicate the existance of at least three different distribution systems for manganese in plasma, and we are curious to know whether their destinations in the tissues are the same or different.

You might have anticipated the question whether the artificial isotope manganese 54 truly reflects the biological behavior of the natural manganese 55. In order to answer this with reference to blood or plasma, we had to use a method capable of measuring the concentration of manganese 55 in these important fluids. We had guessed from the isotopic studies that manganese must be about one thousand times more concentrated in an organ such as the liver than it is in plasma. All reagents tested contain manganese which would contaminate plasma samples (11) which is probably why various chemical methods, we tried at first, only discouraged us. Therefore, we developed a method based on neutron activation analysis which is both precise and accurate enough to within about $\pm$ 3.4% when used on 0.2 ml of plasma. This method does not induce contamination. When plasma from various normal persons was analyzed with this method, a rough average of 2.5 µg of manganese per liter

of plasma was found. In the case of spinal fluid, the concentration was half as much. In both instances, however, a remarkable constancy from person to person became evident, which confirmed our impression that the metabolism of manganese is under homeostatic control. As you recall, this impression was gained first on the basis of an injected radioisotope to metabolic loads with its stable isotope, as revealed by the total body counter. Let me refresh your memory by showing you some observations on a patient (23). This woman had been given Mn^{54} by injection and her total body counts were followed thereafter. After one set of observations the test was repeated but this time manganese sulfate was fed by mouth. On the next slide (Fig. 12) you see the change in her total body radioactivity burden as a function of time. This is again a response similar to the one you saw earlier in animals. Now, let's look at another essential trace element, namely zinc, in the same way, but in the mouse. The next slide (Fig. 13) shows the change of the total body radioactivity burden of Zn^{65} as a function of time and as altered by various oral metabolic loads with nonradioactive zinc sulfate. Here again, radiozinc is being chased out of the body by stable zinc (12, 13).

In the same way, now let us look at an element, the role of which is under considerable scrutiny at present, cadmium, on the next slide (Fig. 14). Here are animals that had received one cadmium 109 injection. Some of them were fed various amounts of cadmium sulfate by mouth (14). As opposed to the two essential elements, which I showed you earlier, this one does not seem to move out of the body either spontaneously or as a consequence of feeding a stable cadmium salt. It would be interesting to test further whether this lack of response might not constitute a criterion of essentiality for various metals.

For many body constituents, the prime end-organ of excretion and of homeostatic control is the kidney. Most of the essential trace metals are excreted primarily or exclusively via the gastrointestinal tract. Manganese must be excreted exclusively via that route, since ligation of the anus will eliminate the loss of its radioisotopes from the bodies of mammals (9). Furthermore excretion of this element into the bile and into the pancreatic juice has been observed by others (15). It should be recalled that the mammalian liver and pancreas are the phylogenetic successors of the hepatopancreas, which is a primordial homeostatic end-organ relative to which the kidney is a newcomer. If the mammalian hepatopancreatic successors were capable of controlling the flux of a primordial metabolite such as an essential trace metal, this might indicate that these successors have maintained their homeostatic adequacy in spite of the advent of the kidney. In a report now in press (16), we have argued that this is indeed the case. In the next slide (Fig. 15) you will see one of the experiments on which this conclusion is based; Rats with and without biliary obstruction were tagged with Mn^{54} and their total body turnover was observed. After it was established that both groups lose their radioisotope, albeit at different rates, half of the animals in each group received supplements of stable manganous sulfate in their diet. When the bile duct was obstructed, the characteristic acceleration of the total body turnover was absent, while it was obviously present in sham operated controls. Furthermore, the controls had received about one half of the manganous supplement of that offered to the animals with biliary obstruction. This and several other experiments convinced us that the flux of manganese through the body is controlled by the bile-producing mechanisms.

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Since homeostasis is controlled by hormones, it became interesting to see whether hormonal effects would reflect themselves on the metabolism of these trace minerals. The concentration of mineral constituents of the body is regulated by and large by the mineralcorticoid hormones of the adrenal gland. Therefore we tested some of these hormones for their effects on the metabolism of manganese. To our dismay, we detected no effects attributable to mineralcorticoid action. Instead of abandoning the adrenal hormones all together, we turned to the so-called glucocorticoid hormones, namely the ones which act primarily on organic constituents such as glucose. To our delight, the metabolism of manganese as tested in intact mice, appeared to respond quite definitely to the action of these hormones (17). We were happy with this result because cortisone and its cogeners are indeed medically important for two reasons: First, under conditions of stress, surgery, trauma, shock, or infection, one of the most immediate responses of the body is the hypersecretion of these cortisone-like hormones by the adrenal gland. Second, cortisone and its congeners are of extensive and often of lifesaving utility in today's practice of medicine.

Surgical stress was reflected in the excretion of manganese in the bile. Both natural and artificial isotopes of manganese behaved in the same fashion. This indicated that the information gained with the aid of an artificial manganese isotope reflected the metabolism of the natural isotope. I will show you only the pattern of a surgically stressed animal. A nonstressed animal excretes its radiomanganese asymptotically into its bile. The data shown on the next slide (Fig. 16) were collected from a rat under ether anesthesia with its abdomen open and with bile being collected from a catheter in the common bile duct. A dose of manganese 54 had been given intravenously

at the outset. Both the radioactive and the natural manganese isotopes were quantitated and the results were expressed per unit weight of bile. While the specific activity of manganese 54 tended to reach equilibrium, the absolute amounts of both species of manganese first declined and then increased suddenly.

Thereafter, we searched for concordant changes in patients who needed to be treated with cortisone-like hormones, to combat some underlying disease. Among the many diseases for which cortisone therapy is indicated, we chose to study the collagen diseases such as arthritis and lupus erythematosus disseminatusg We have studied 6 cases of rheumatoid arthritis with cortisone like hormones and in all of them we found the lchange for which we were seeking. The evidence was collected by means of surface scanning, total body counting, and neutron activation analysis of plasma. These experiences are illustrated by one case shown on the next slide (Fig. 17).

Here you see the behavior of intravenously injected manganese 54 after administration of prednisone. The total body radioactivity and that of the liver declined sharply following the institution of treatment, pretty much as you saw in the case of the woman to whom we gave manganous sulfate by mouth. The curve of the radioactivity in mid-thigh, shows a slight decline in the beginning, which stopped when the steroid hormone was given. This could happen if manganese were reaching the mid-thigh from the other parts of the body. The most probable way for manganese to do so is through the bloodstream. Therefore, let's look at the manganese level in this patient's plasma on the next slide (Fig. 18). Here you see again the change in the total body radioactivity level brought upon by cortisone treatment in association with which there occurred a marked rise in the concentration of natural manganese in the patient's plasma.

Our most recent work, which is still in progress, might well give us a powerful lead in our study of extrapyramidal diseases. These are the diseases with which I started this talk. Earlier we had studied some drugs which are useful in the treatment of these diseases, referred to collectively as phenothiazine drugs. One of them is the popular tranquilizer thorazine. Solutions of these drugs in water would react with some biologically important trace elements, including manganese (18). The products of these reactions were highly colored metastable free radicals. We attempted to explain the pharmacological effect of these drugs on the basis of free radicals formed by interaction with trace elements (19). There is a drawback to this theory, namely that we had observed these interactions only in a test tube, not in a living organism. I have been concerned with the fact that we had to show <u>some</u> correlation between <u>some</u> free radical and a trace element like manganese, in <u>some</u> kind of biological system before we could take our theories seriously. Frankly, I became willing to settle for any free radical and postpone the search for a phenothiazine free radical in a biological system, provided that manganese could be linked to the site of production of such a free radical. Fortunately, an article appeared from Southampton, England (20) which indicated that the color of human hair correlated with the amount of melanin free radicals in their hair. Soon another article came out from Bethesda, Maryland (21) which said that melanin from the eye shows free radicals when hit by light. Melanin is not a pheonthiazine but phenothiazines are known to increase melanogenesis. It might be of some value therefore if manganese could be correlated with melanin. Hence we tested whether hair of various shades of darkness, which had already been shown to contain commensurate amounts of free radicals, would contain concordant amounts of manganese. The preliminary results are

shown in the next slide (Fig. 19). Not only did we test hair from various species but we also tested bird feathers (22). The results with one multicolored feather are much more convincing than results with many multicolored hairs, because hairs have individual follicles, while barbs of feather have one. Note that the concentration of manganese in colored hair or feathers is always higher than in white colored structures. Experiments with bovine conjunctivae were concordant. We interpret this as meaning that manganese cannot be excluded as playing a similar role in melanogenesis as it had in the formation of phenothiazine free radicals. This leads back to the extrapyramidal diseases with which we started (24).

I am told by neuropathologists that discoloration of the substantia nigra of the brain, in other words, the loss of black pigment from the substantia nigra, is about the only constant finding at autopsy in cases suffering from Parkinsonism in life. This pigment contains free radicals, metals and several metabolic end products from the metabolism of neurotropic amines. We are now engaged in testing whether alterations in these granules might be responsible for the similarities exhibited by many extrapyramidal diseases of different etiology.

SUMMARY

The recent development of sharp analytical resolution has permitted world-wide initiation of integrated studies of the kinetics, the physical state and the chemical behavior of trace metals in relation to their concentration. Nonetheless, it had already become evident that these metallic nutrients might be more essential to life than even oxygen, since only some living things require the latter while all of them seem to require the former.

Two essential metals, manganese and zinc and a non-essential one, cadmium, are followed through considerable portions of their respective pathways through the body. The pathways are examined with regard to their biological specificity. Attention is given to the role of the liver and other tributaries of the gastrointestinal system as surrogate kidneys in homeostasis. Methodology is touched upon with emphasis on isotopes and the analytical potential of nuclear reactors. Several distortions of homeostatic regulation are considered with regard to their nutritional, clinical, and biochemical meaning. The homeostatic role of some drugs is indicated.

Polyvalent elements must have been operating prior to the Darwinian evolution of macromolecules. The implications of this statement are detailed.

FOOTNOTES

1. The scientific work presented here was done in collaboration with the following: L.S. Maynard; D.C. Borg; A.J. Bertinchamps: P. S. Papavasiliou: E.R. Hughes: R.L. Golden: A. Sakamoto. Technical assistance was provided by: J.J. Greenough: H. Hamel: B. Selleck: J. Gulibon: S.T. Miller: and C.A. Rozanski. Miss Martha Hill, R.N. has expertly supervised the Metabolic Ward.

2. The forms of manganese poisoning mimic Wilson's Disease. Parkinsonism andtthe schizophrenia-like psychoses respectively.

LEGENDS TO FIGURES

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

Figure 2: Distribution of Mn^{56} among rat organs, Recalculated from Reference No. 1. (see text).

Figure 3: Intracellular distribution of Mn^{56} recalculated from Reference No. 1. (see text).

Figure 4: By permission, the Editors of the Journal of Clinical Investigation. (see text).

Figure 5: By permission, the Editors of the Journal of Clinical Investigation. (see text).

Figure 6: By permission, the Editors of Nature. (See text).

Figure 7: A schematic drawing of blood fractionation discussed in text.

Figure 8: Mouse counter, by permission J. B. Lippincott Co., Philadelphia.

Figure 9: By permission, the Editors of the Journal of Clinical Investigation. (see text).

Figure 10: By permission, the Editors of the Journal of Clinical Investigation. (see text).

Figure 11: Zone electrophoresis of plasma containing the indicated concen- of Mg^{++}: The Mn^{54} concentration of only two electrophoretic fractions is shown from two separate experiments.

Figure 12: By permission, J. B. Lippincott Company, Philadelphia (see text).

Figure 13: By permission, the Editors of the American Journal of Physiology. (see text).

Figure 14: By permission, the Editors of the American Journal of Physiology. (see text).

Figure 15: Total body turnover of Mn^{54} in rats: A and B - rats with biliary obstruction; only group B received carrier (Mn^{55++}, 2.2 γ per ml of milk). C and D - laparotomized rats; only group D received carrier (Mn^{55++} 1.1 γ per ml of milk).

Figure 16: Change in the concentration of Mn^{54}, Mn^{55} and specific activity in the bile of a rat under surgical stress.

Figure 17: Change of the Mn^{54} concentration in the whole body, the liver and the mid-thigh of a woman receiving prednisone.

Figure 18: Same as Figure 17 but with plasma manganese concentration plotted.

Figure 19: The ratio of manganese concentration of various pigment structures, relative to their light colored or nonpigmented controls. Only hair and feathers are shown.

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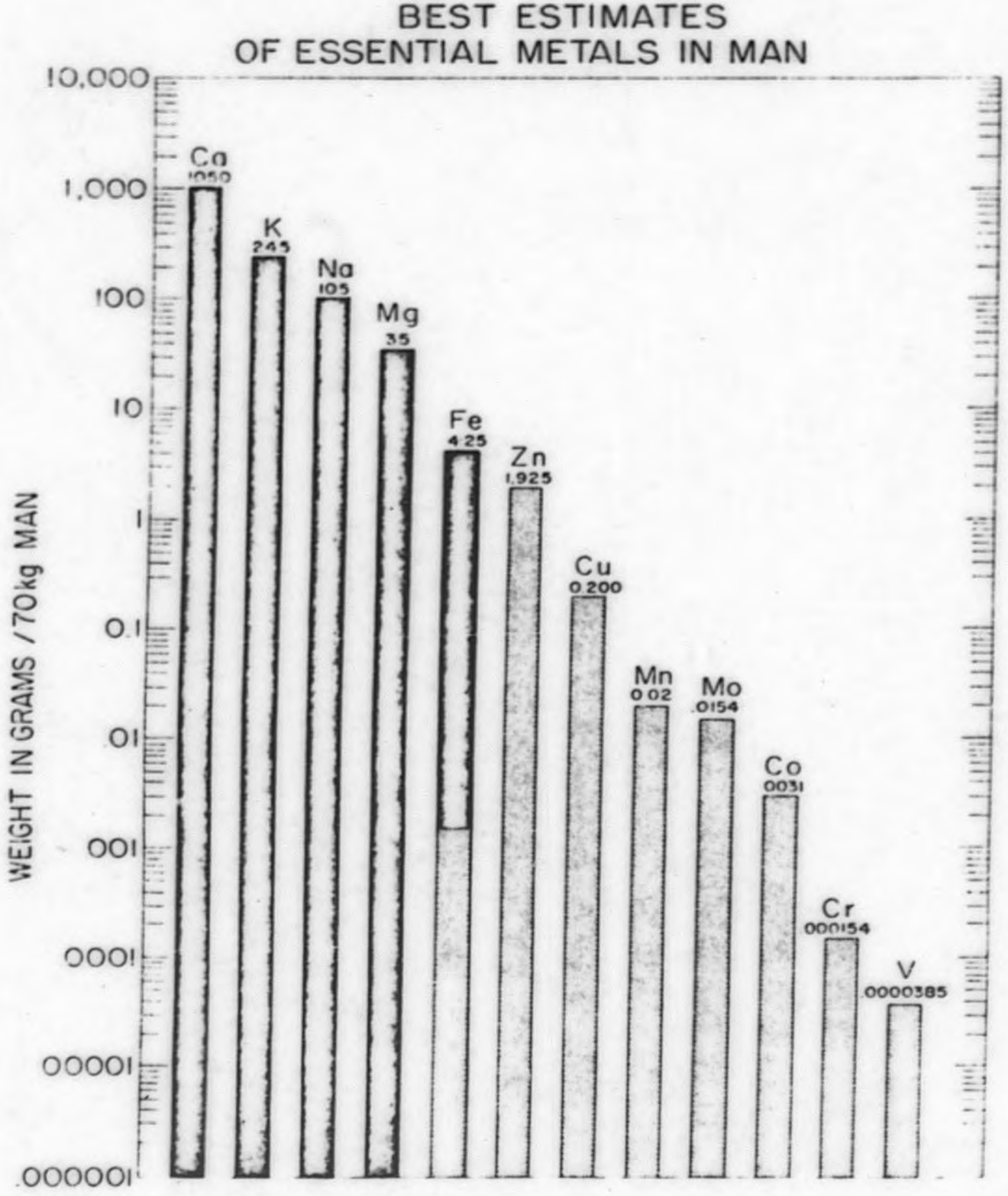

BEST ESTIMATES
OF ESSENTIAL METALS IN MAN
WEIGHT IN GRAMS / 70kg MAN
10,000
1,000
100
10
1
0.1
01
001
0001
00001
.000001
Ca
1050
K
245
Na
105
Mg
35
Fe
4.25
Zn
1.925
Cu
0.200
Mn
0 02
Mo
.0154
Co
0031
Cr
000154
V
.0000385

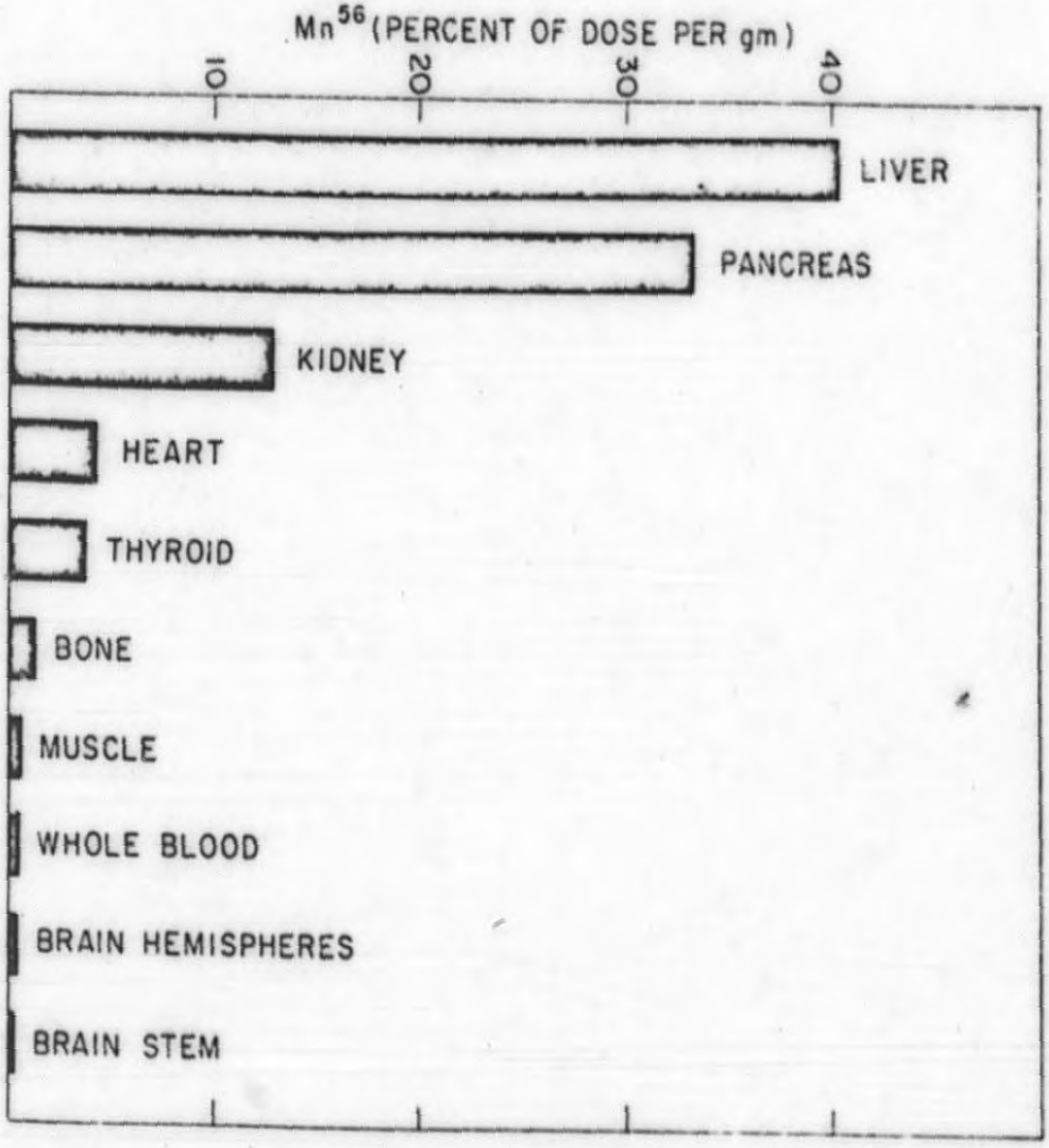

Mn56 (PERCENT OF DOSE PER gm)
10
20
30
40
LIVER
PANCREAS
KIDNEY
HEART
THYROID
BONE
MUSCLE
WHOLE BLOOD
BRAIN HEMISPHERES
BRAIN STEM

FIGURE 2

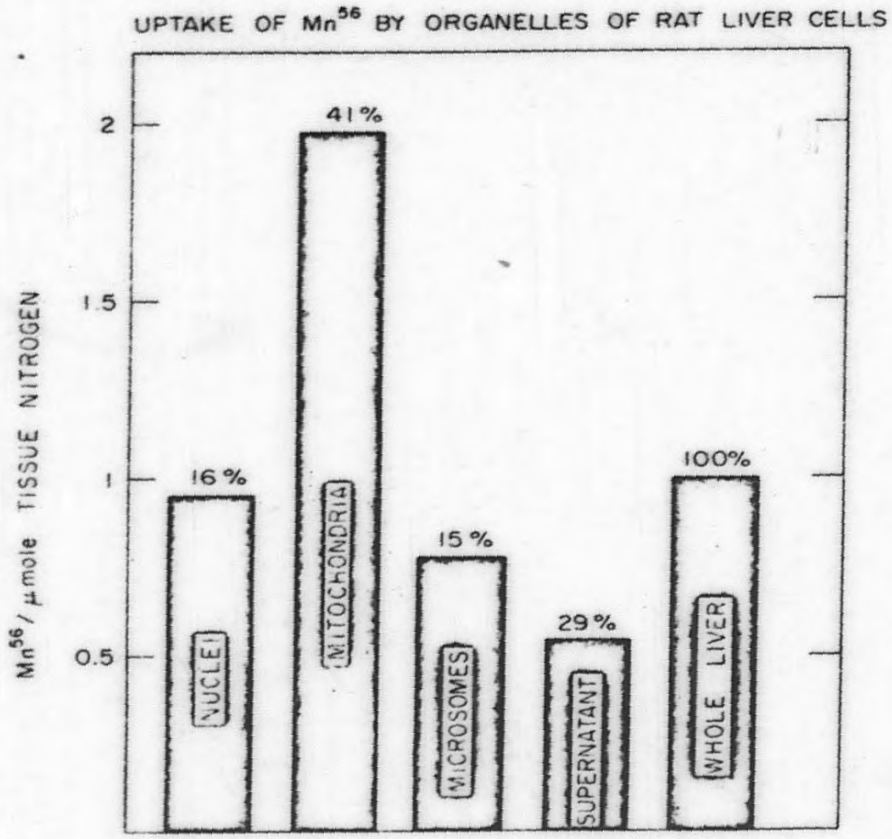
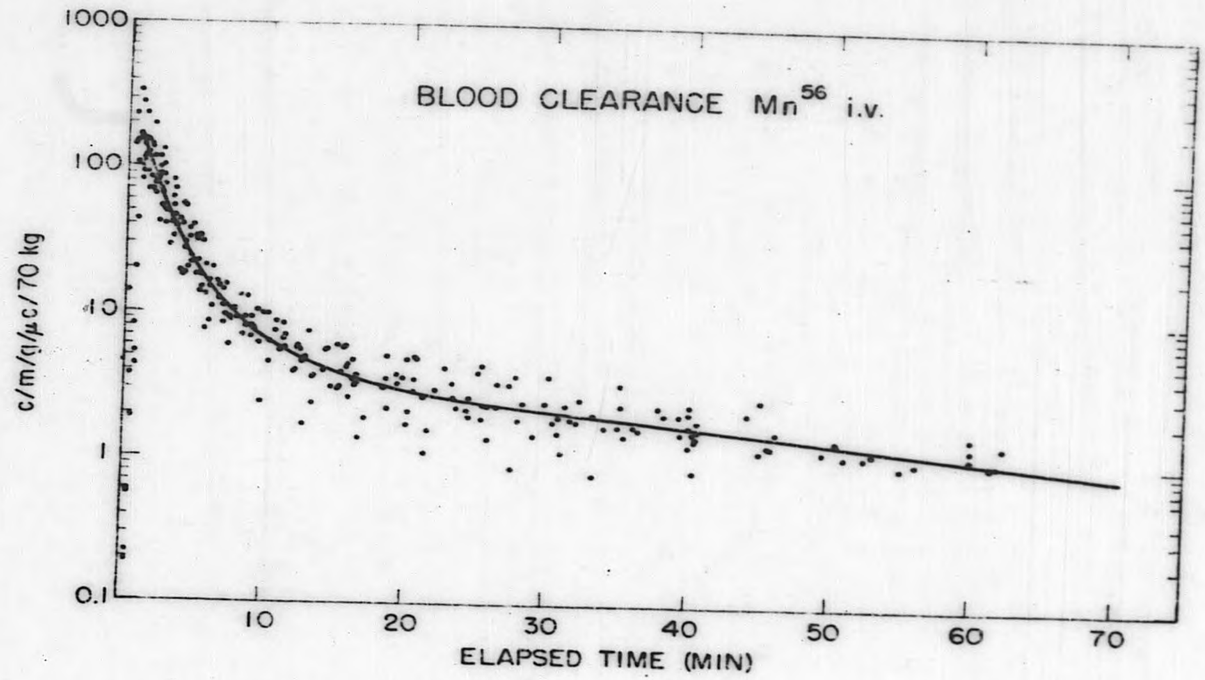

UPTAKE OF Mn^{56} BY ORGANELLES OF RAT LIVER CELLS
Mn^{56}/μmole TISSUE NITROGEN
2
1.5
1
0.5
41%
16%
15%
29%
100%
NUCLEI
MITOCHONDRIA
MICROSOMES
SUPERNATANT
WHOLE LIVER

FIGURE 3

FIGURE 4


BLOOD CLEARANCE Mn56 i.v.
c/m/η/μc/70 kg
1000
100
10
1
0.1
10
20
30
40
50
60
70
ELAPSED TIME (MIN)

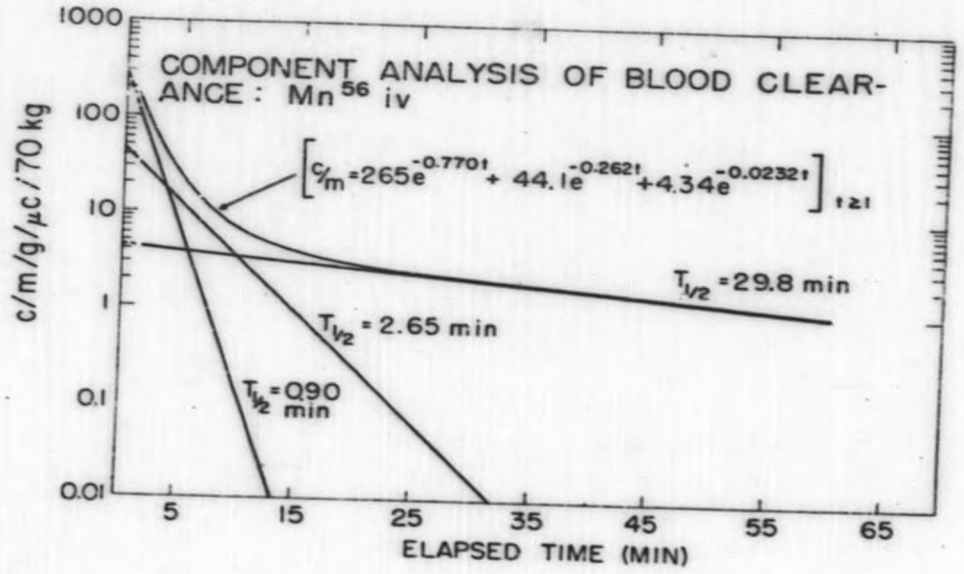
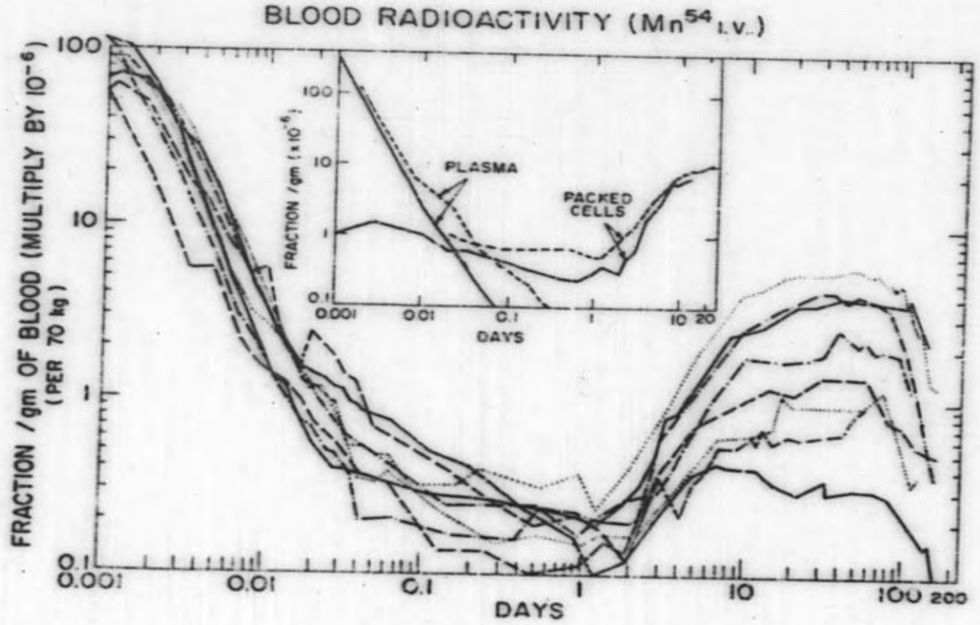

COMPONENT ANALYSIS OF BLOOD CLEARANCE : Mn 56 iv
$\left[C/m = 265e^{-0.7701t} + 44.1e^{-0.2621t} + 4.34e^{-0.0232t}\right]_{121}$
$T_{1/2}$ = 29.8 min
$T_{1/2}$ = 2.65 min
$T_{1/2}$ = 0.90 min
c/m/g/μc/70 kg
1000
100
10
1
0.1
0.01
5
15
25
35
45
55
65
ELAPSED TIME (MIN)

FIGURE 5


BLOOD RADIOACTIVITY (Mn^{54} I.V.)
FRACTION /gm OF BLOOD (MULTIPLY BY 10^{-6})
(PER 70 kg)
100
10
1
0.1
0.001
0.01
0.1
1
10
100
200
DAYS
FRACTION /gm (x 10^{-6})
PLASMA
PACKED CELLS
0.001
0.01
0.1
1
10
20
DAYS

BLOOD FRACTIONATION
(* = Mn^{54})

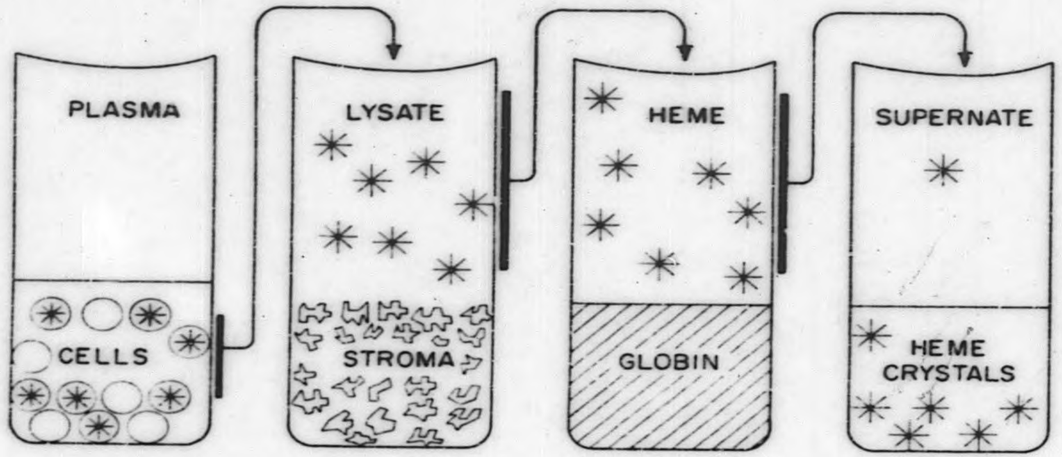


FIGURE 7

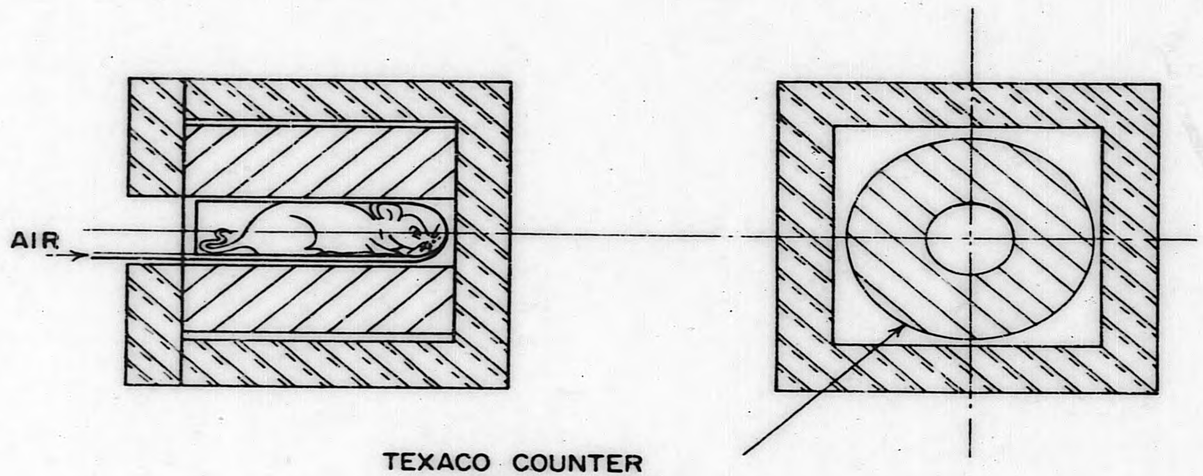


FIGURE 8

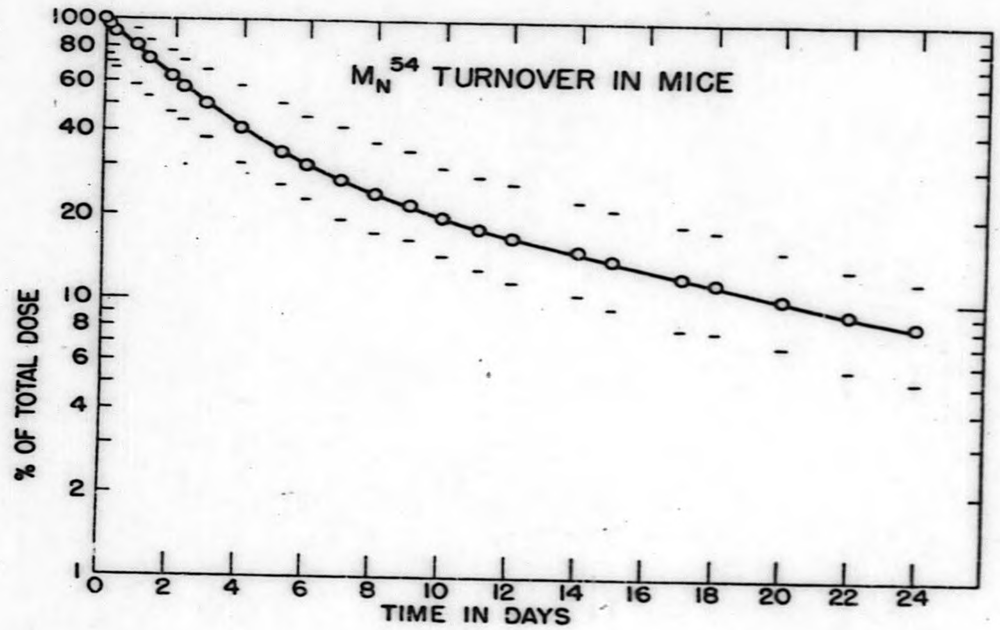

M_N^{54} TURNOVER IN MICE
% OF TOTAL DOSE
100
80
60
40
20
10
8
6
4
2
1
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME IN DAYS

FIGURE 9

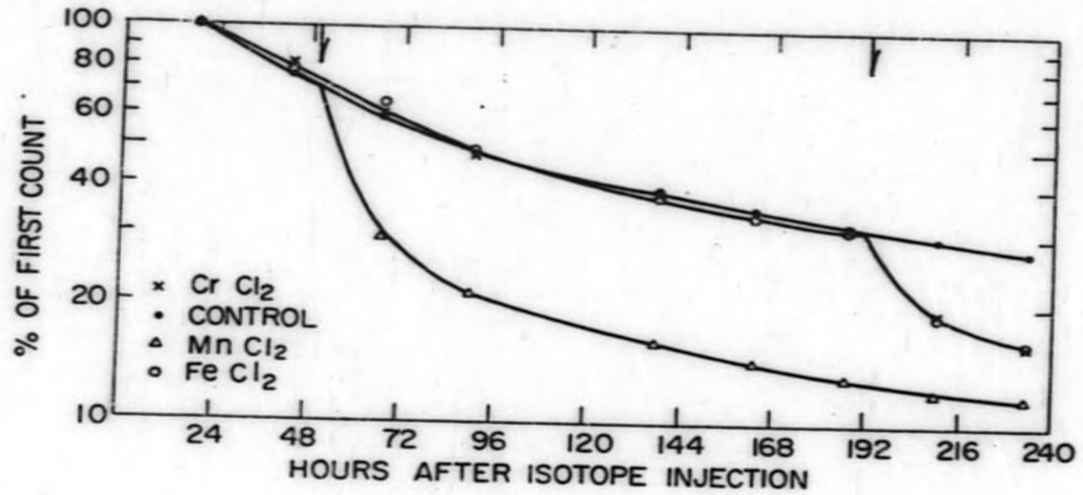

100
80
60
40
20
10
% OF FIRST COUNT
× Cr Cl2
• CONTROL
△ Mn Cl2
○ Fe Cl2
24
48
72
96
120
144
168
192
216
240
HOURS AFTER ISOTOPE INJECTION

FIGURE 10

FIGURE 11

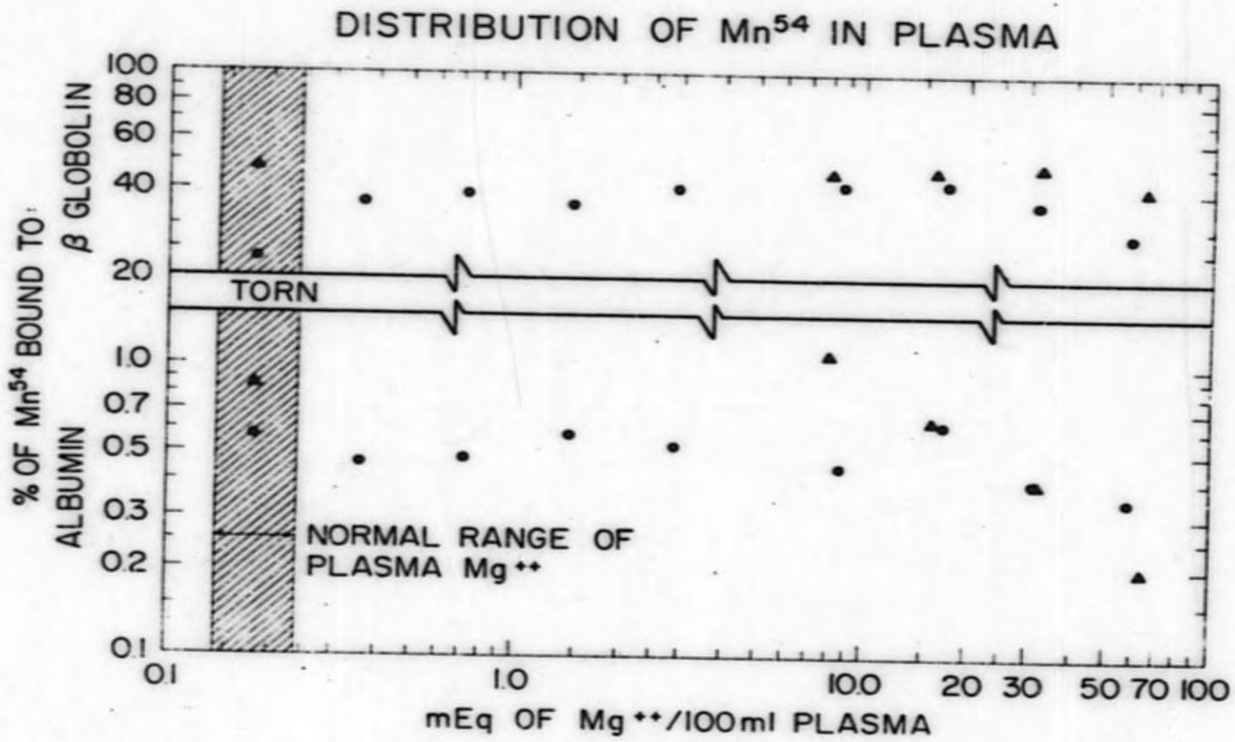
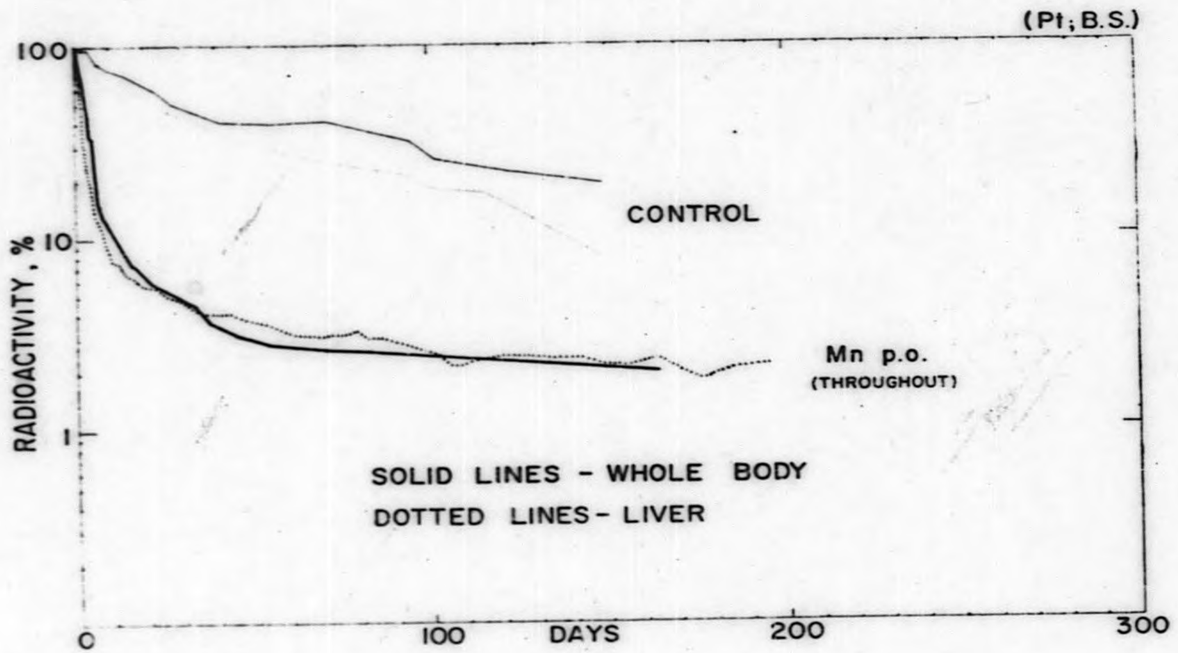

DISTRIBUTION OF Mn54 IN PLASMA
% OF Mn54 BOUND TO:
ALBUMIN
β GLOBOLIN
100
80
60
40
20
10
0.7
0.5
0.3
0.2
0.1
TORN
NORMAL RANGE OF
PLASMA Mg++
0.1
1.0
10.0
20
30
50
70
100
mEq OF Mg++/100ml PLASMA

FIGURE 12


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

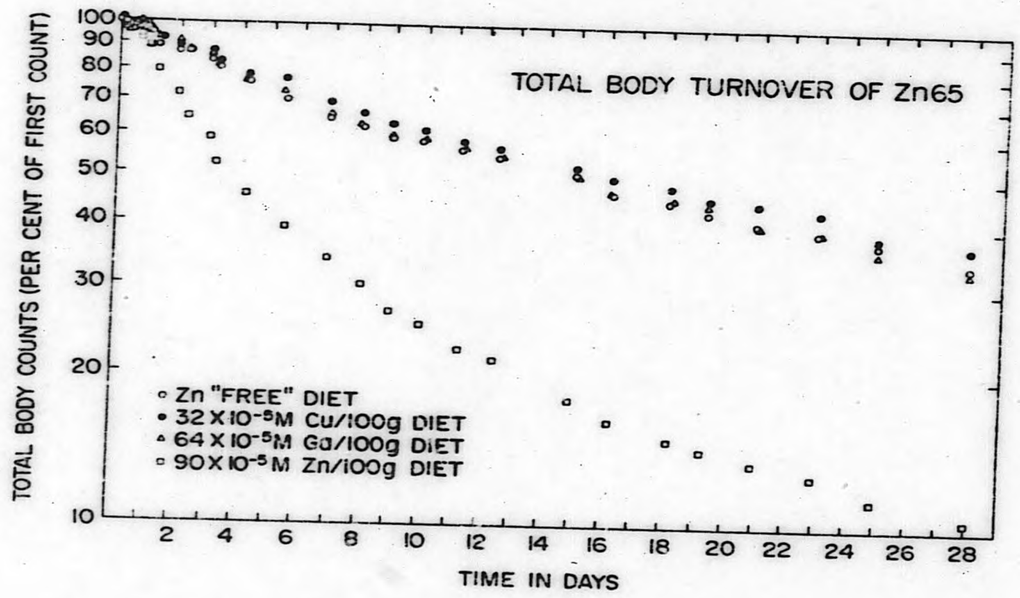
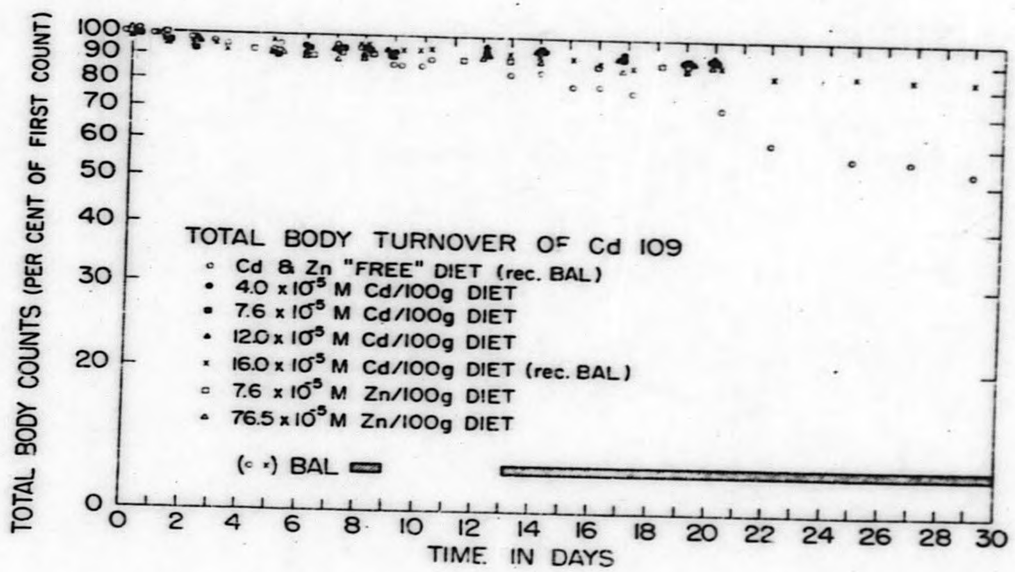

TOTAL BODY TURNOVER OF Zn65
TOTAL BODY COUNTS (PER CENT OF FIRST COUNT)
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME IN DAYS
○ Zn "FREE" DIET
● 32 X 10⁻⁵M Cu/100g DIET
▲ 64 X 10⁻⁵M Ga/100g DIET
□ 90 X 10⁻⁵M Zn/100g DIET

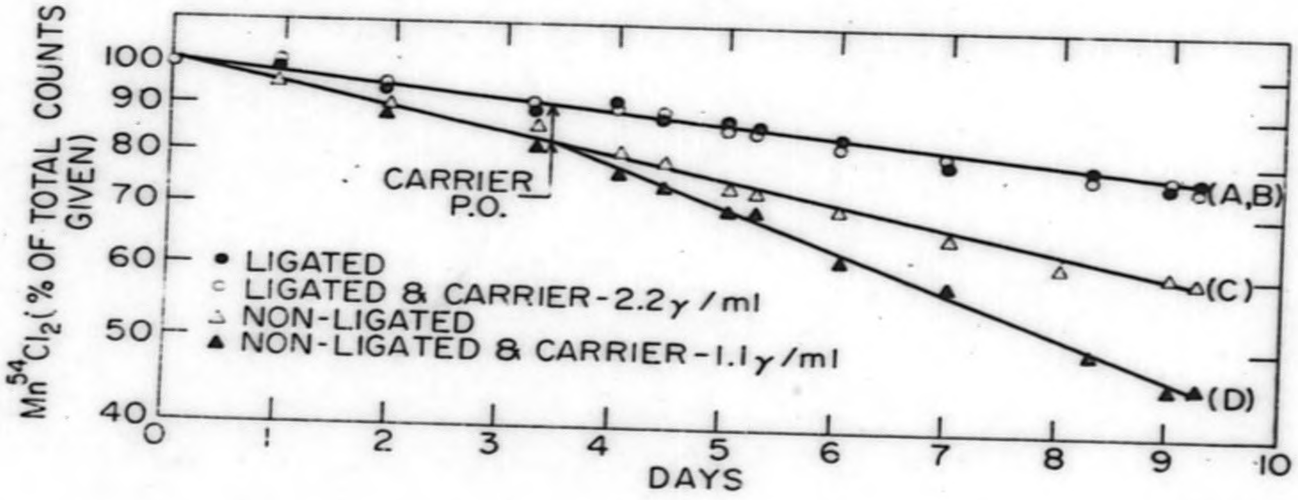
FIGURE 13

FIGURE 14


TOTAL BODY COUNTS (PER CENT OF FIRST COUNT)
100
90
80
70
60
50
40
30
20
0
TOTAL BODY TURNOVER OF Cd 109
Cd & Zn "FREE" DIET (rec. BAL)
4.0 x 10⁻⁵ M Cd/100g DIET
7.6 x 10⁻⁵ M Cd/100g DIET
12.0 x 10⁻⁵ M Cd/100g DIET
16.0 x 10⁻⁵ M Cd/100g DIET (rec. BAL)
7.6 x 10⁻⁵ M Zn/100g DIET
76.5 x 10⁻⁵ M Zn/100g DIET
(• ×) BAL
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME IN DAYS

12-95-64

FIGURE 15



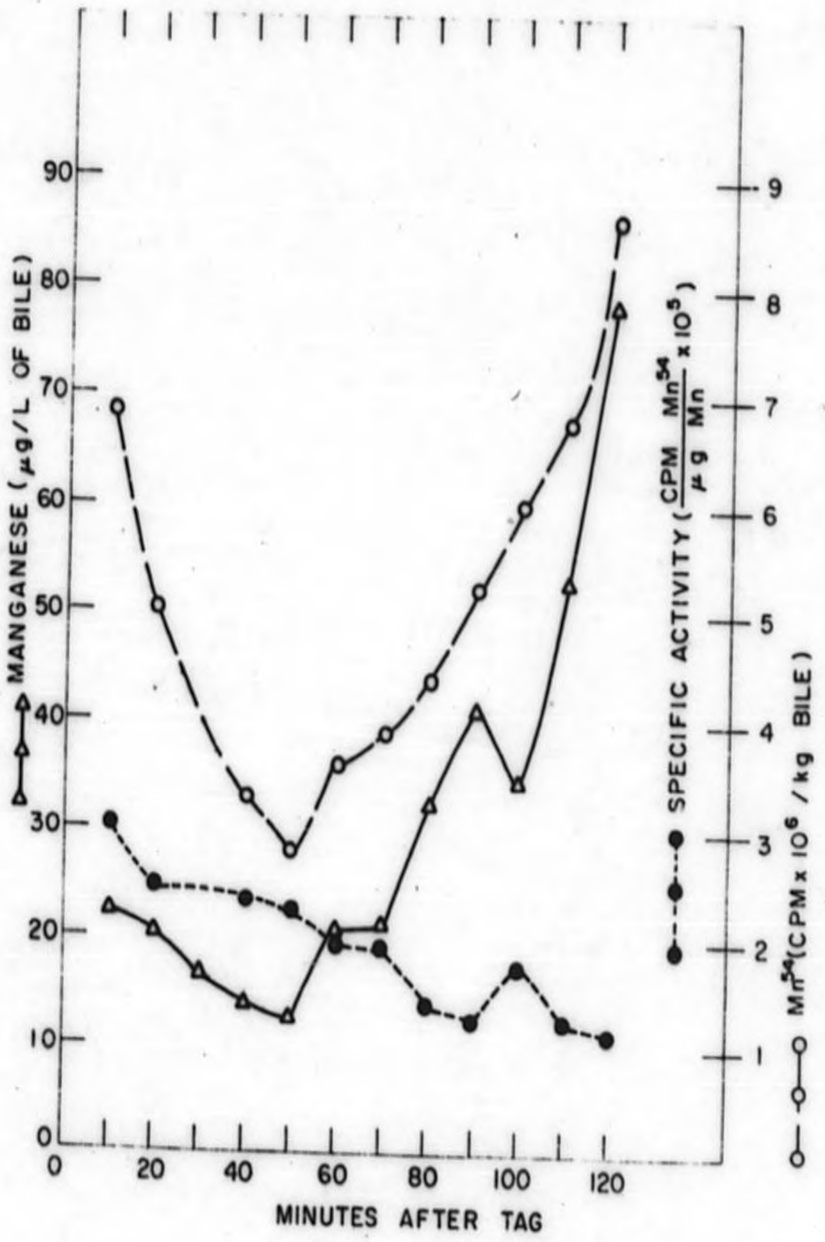

MANGANESE (μg/L OF BILE)
SPECIFIC ACTIVITY ($\frac{CPM\ Mn^{54}}{\mu g\ Mn} \times 10^3$)
Mn^{54} (CPM x 10^6 / kg BILE)
MINUTES AFTER TAG
0
10
20
30
40
50
60
70
80
90
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
120

FIGURE 16

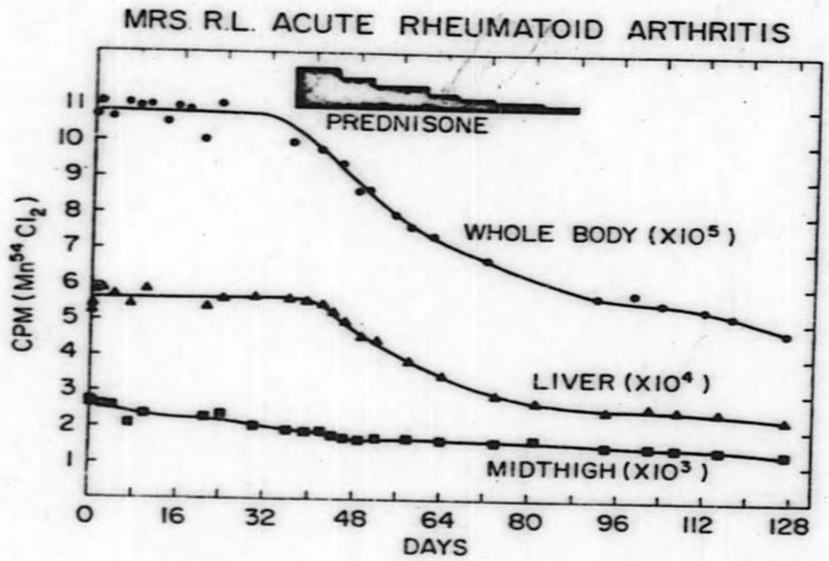

MRS. R.L. ACUTE RHEUMATOID ARTHRITIS
PREDNISONE
WHOLE BODY (X10⁵)
LIVER (X10⁴)
MIDTHIGH (X10³)
CPM (Mn⁵⁴Cl₂)
11
10
9
8
7
6
5
4
3
2
1
0
16
32
48
64
80
96
112
128
DAYS

FIGURE 17

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
1
2
3
4
5
6
7
8
9
10
11

FIGURE 18

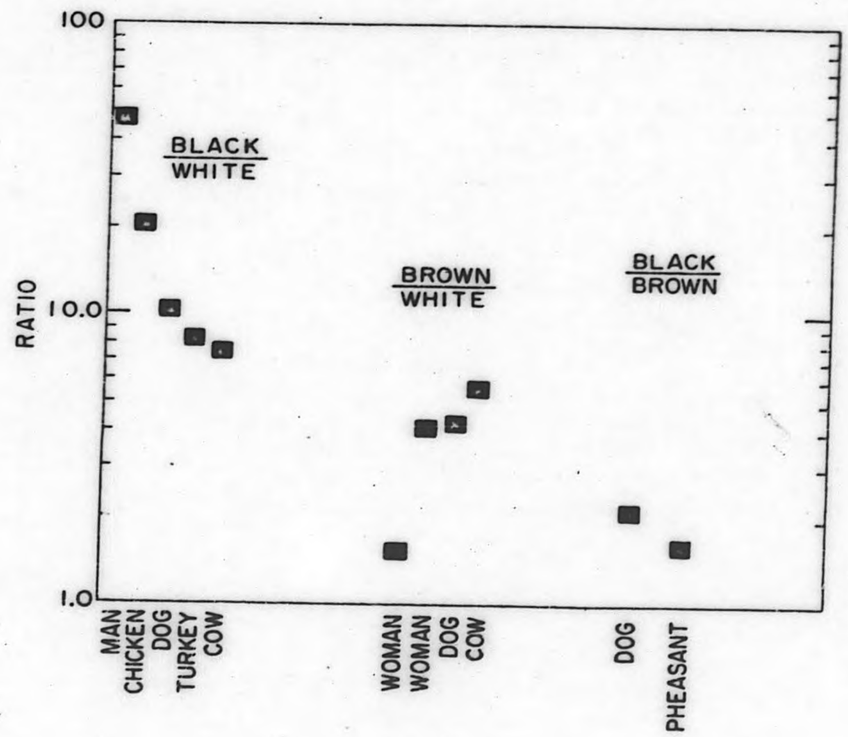


FIGURE 19

END