Distribution and Fate of ^{54}Mn in the Monkey: Studies of Different Parts of the Central Nervous System and Other Organs

DARAB K. DASTUR, DAYA K. MANGHANI, and K. V. RAGHAVENDRAN

From the Neuropathology Unit, Grant Medical College and J. J. Group of Hospitals, Bombay-8 and the Radiation Medicine Centre, Tata Memorial Hospital, Bombay, India

ABSTRACT The fate and distribution of isotopic manganese administered as a single carrier-free dose of 200 μCi of maleate-^{54}Mn to 12 rhesus monkeys was studied at different time periods from the 6th hr to the 278th day. Whole-body activity was measured, and all body organs and tissues and different parts of the central nervous system (CNS) were evaluated for specific activity, exponential analysis, and relative retention. Exponential analysis revealed a pattern of discharge with a fast and a slow component for the whole body and for many of the viscera. All parts of the CNS and, to a lesser degree, the thyroid and muscle showed an almost steady state of activity after the initial uptake. While the whole body and most organs and tissues appeared to discharge their radioactivity with the passage of time, first rapidly and then gradually, the CNS, endocrine glands, and muscle tissues showed persistent levels of specific activity. All components of the brain exhibited increasing relative retention, the lentiform nucleus and the cerebellum showing this more. It is suggested that the selective vulnerability of the brain in manganese miners might result from this inability on the part of the CNS to discharge the ^{54}Mn with time. This investigation confirms and amplifies our earlier similar study on the rat.

INTRODUCTION

A small but significant proportion of the dry drillers at the mines in India (1), Chile (2), and in other countries present with an extrapyramidal cerebral disorder reminiscent of parkinsonism (3). While detailed neuropathological accounts are not available, some of the autopsy reports suggest damage to the globus pallidus (3, 4). Furthermore, although it has been difficult to induce experimental manganism, Van Bogaert and Dallemagne (5) reported cerebellar damage in a monkey subjected to inhalation of MnO_2, and Pentschew, Ebner, and Kovatch (6) produced neuronal degeneration and glial cell reaction in another after intraperitoneal injection. In our earlier observation over 3 yr,[1] only 1 of 15 monkeys repeatedly injected over several months with intraperitoneal MnO_2 or $MnCl_2$ developed a basal ganglionic disorder. This took the form of an intense and increasing restlessness which finally led to incessant jerky choreic movements of limbs rendering him unable to eat or sleep and leading to death in 3 wk. No clear-cut histological lesion could be detected in the brain, although there was some pyknosis of neurons of the globus pallidus and the putamen, with some sponginess of the latter. However, this feature was also noticed in another animal who did not manifest any clinical disorder. Moreover, there was no correlation between the level of manganese (chemically detected) in the basal ganglia or in the brain and the production of the neurological disorder. Thus, consistent with the earlier suggestion of Cotzias (7), our own observations led us to suspect an essentially chemical lesion in human and experimental manganism, possibly at the level of intracellular micelles.

In the report of the first part of this study, it was indicated that there is a paucity of information on the pattern of distribution of radioactive or stable manganese in the various organs and tissues, especially

The material included here formed part of a Guest Lecture on "two heavy metals in the brain," delivered by the first author at the National Institutes of Health, Bethesda, Md., and the National Hospital for Nervous Diseases, Queen Square, London, in October 1969.

Received for publication 2 March 1970 and in revised form 7 July 1970.

[1] Dastur, D. K., and D. K. Manghani. Unpublished data.

long after its administration, and a lack of understanding on the pathogenesis of selective vulnerability of the brain in chronic manganism in man. Therefore, the fate and distribution of maleate-^{54}Mn were studied in the rat over a period of time extending from 15 min to 34 days (8). A lack of discharge of the metal from the CNS resulting in its relatively greater retention in this tissue through the 5 wk, was observed. In the second part of this investigation on 12 additional rats, the effect of a small dose (less than 2 mg) of stable manganese administered 3 days after the ^{54}Mn was studied up to the 64th day. It was observed that this load failed to produce any increase in discharge of the isotopic manganese from the brain and that the relative retention here at the 64th day was even greater than at the 34th (9).

The present investigation was therefore undertaken on the monkey (*a*) to confirm the observations in the rat on a larger mammal, and (*b*) to study the distribution of ^{54}Mn in different parts of the brain, keeping in mind the basal ganglionic disturbance which is such a prominent feature of the human disorder in manganese miners (10).

METHODS

The same isotope, carrier-free $^{54}MnCl_2$ with a half-life of 290 days and a gamma energy of 0.835 Mev as was used previously (1), was converted to a maleate-^{54}Mn solution with pH of about 7.5. 12 Rhesus monkeys weighing 2.5 kg on an average (range of 2.0–3.4) were used. A dose of 200 μCi of maleate-^{54}Mn contained in 4.0–6.0 ml volume was injected intraperitoneally into each animal at the start of the experiment.

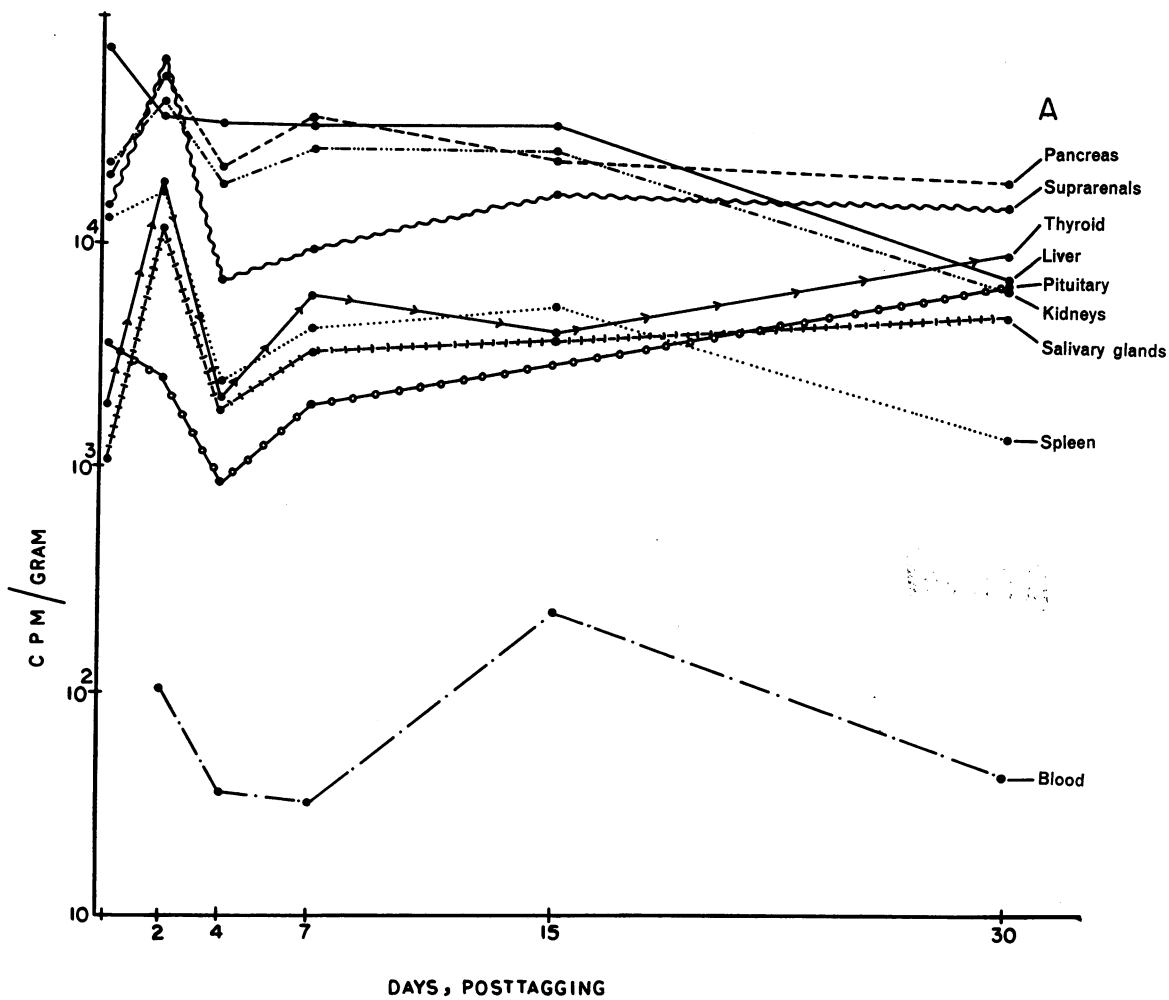


FIGURE 1 (A and B) Specific activity of radiomanganese plotted on 3 cycle log paper, from the 1st to the 30th day in (A) and from the 7th to the 278th day in (B), for the more cellular organs and the blood. Note the very low level in the latter throughout the greater part of the experiment.

The animals were sacrificed, one each, after 6 hr, and after 2, 4, 7, 15, 30, 50, 70, 100, 165, 222, and 278 days. The whole-body activity was measured before they were killed by placing the anaesthetized animal 100 cm away from two probes of NaI (T_1) crystals, each $2 \times 1\frac{1}{2}$ inches, kept 30 cm apart and connected to two single-channel analyzers and scalers. The system was designed to count independently of geometry and was quite sensitive. An amount corresponding to a single dose of injected radioactive material, viz., 200 μCi of ^{54}Mn made up to 100 ml with dilute HCl, was used as a standard solution for the whole-body counting. This amount was maintained in two volumetric flasks of 50 ml each, and their combined radioactivity was measured by keeping them 30 cm apart and 100 cm away from the paired crystals. 1 ml of this solution was taken for the well-type counter as the standard for individual organs. The activity in this original standard was measured each time along with the whole body or organs of the monkey in question so that the decay in radioactivity with time was comparable in the standard and in the tissues. In addition to all the organs and tissues counted in the rat (8), different parts of the brain, i.e. grey and white matter, respectively, lentiform nucleus, caudate nucleus, and thalamus separately, and the brain stem were counted separately in a well-type scintillation detector with a $3 \times 2\frac{1}{2}$ inch NaI (T_1) crystal connected to a gamma ray spectrometer. The activity in the carcass, consisting essentially of bones, muscles, and skin with hair, was measured by the paired scalers used for the whole-body counting.

In 9 of the 12 monkeys, biopsy of the liver, muscle, and skin with hair was carried out once, 1, 3, 7, 23, 38, 61, 85, 144, and 184 days, respectively, after the injection. This provided points of radioactivity at some time before the autopsy in these animals.

As before, the results are given in the form of (*a*) specific activity expressed as counts per minute (cpm) per gram of tissue, (*b*) retained radioactivity in the whole body, expressed as percentage of total dose injected, (*c*) exponential analysis of radioactivity in the whole body and selected tissues, and (*d*) the relative retention of radioactivity expressed as ratio of cpm per gram of tissue per cpm per gram of whole body.

RESULTS

Counts per minute per gram of tissue

The results are illustrated in Figs. 1–3. It is seen that on the basis of uptake and discharge the organs can be roughly divided into three groups. Thus on the whole, like the observations on the rat (8), the highest concentration of activity was observed in the very cellular and

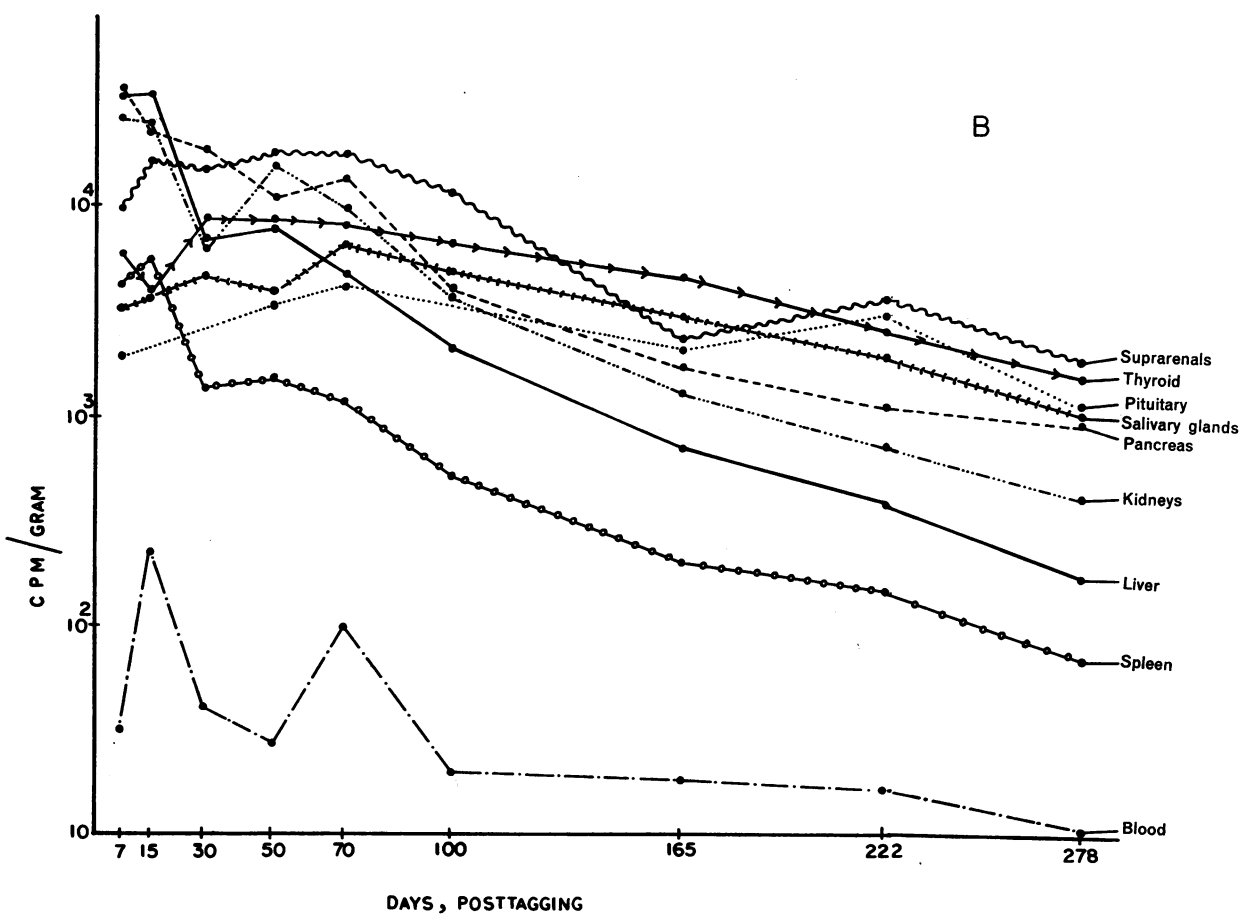


FIGURE 1—(*Continued*)

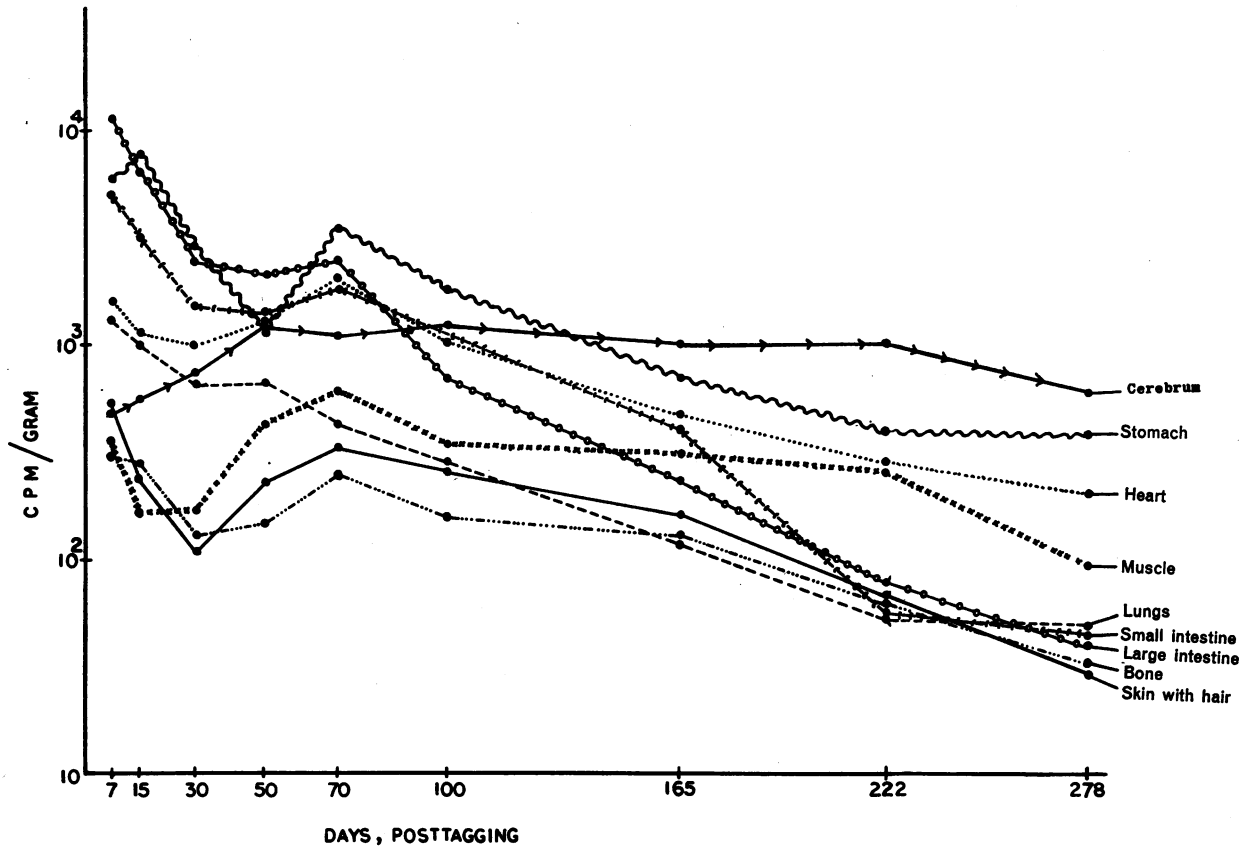


FIGURE 2 Specific activity recorded and expressed as in Fig. 1 B, for other organs and tissues. Note the different behavior of the cerebrum.

glandular structures (Fig. 1). After an initial rise in the first 2 days, the organs of this group showed a fall in radioactivity by the 4th day. On the 7th and the 70th day the organs showed a second and a third peak, respectively, which must be due to absorption from the blood. From the 70th day there was a persistent and more prolonged discharge of the ^{54}Mn by all organs.

The second group consisting of the heart, lungs, and the three constituents of the carcass, namely the bone, muscle, and skin with hair, showed a lower peak concentration (Fig. 2). The pattern of uptake and discharge in these organs was similar to the pattern of the organs of the first group with the exception of the muscle which showed a very slow discharge from the 100th day onward.

The third group comprising the tissues of the central nervous system (CNS), viz., grey matter, basal ganglia, white matter, cerebellum, brainstem, and the spinal cord, showed (Fig. 3), unlike the rest of the organs, a rising or a steady trend of radioactivity from the 7th to the 278th day. The only point of similarity between these and the organs of other groups was the first peak uptake by the 2nd day, the sharp fall by the 4th day, and the second peak by the 7th day (Fig. 3 A). From then on all the components of brain showed a rising or a steady trend of radioactivity. While there were no striking differences in the levels of radiomanganese in the various components of the brain (Fig. 3 B), the cerebellum and basal ganglia showed the highest value at all times. In the later stages of the experiment the different constituents of the basal ganglia, namely the lentiform nucleus, the caudate nucleus, and the thalamus, were dissected. It was seen that among these three the lentiform nucleus recorded the greatest radioactivity, and the thalamus and the caudate nucleus came next, in that order (Fig. 3 B). The peripheral nerve tissue stood apart at a markedly lower level of radioactivity than any of the parts of the CNS (Fig. 3 B).

Blood

Figs. 1 A and B show the pattern and the actual levels of radioactivity in the blood. Like the observations in the rat the activity is seen clearly to be at a lower concentration than in any other organ. Blood could not be collected in the first 6 hr animal. From the 2nd to the 4th day there was a sharp fall of radioactivity in the blood.

The level rose sharply from the 7th day to a second peak on the 15th day, fell till the 50th day (Fig. 1 B), showed a third peak of uptake on the 70th day, and then a rapid fall till the 100th day. It appears important to note that the fall till the 2nd day and the peak activity on the 15th day represent an inverse situation to that obtained in the other organs. From the 100th to the 278th day a very low but perceptible level of radioactivity was maintained by the blood.

Exponential analysis

Whole body. The activity in the whole body (Fig. 4) showed clearly two phases of discharge, an earlier faster and a later slower phase, until the end. Hence, the whole-body distribution of ^{54}Mn could be resolved into two exponential components (Fig. 4). The half-time of discharge through the fast component was about 6 days and the half-time for the slow component was 95 days.

Organs. An exponential analysis was again carried out on selected organs which manifested different peaks of activity and rates of discharge. Thus, for the period from the 15th day on, the liver showed exponential components of $t_{\frac{1}{2}}$ of 13 and 64 days, corresponding, respectively, to the fast and slow discharges. The gastrointestinal tract and most of the viscera showed a two-phase exponential pattern of discharge similar to that for the liver. The thyroid and muscle showed slower growth and decay phases for the same period, with a single discharge pattern and a longer $t_{\frac{1}{2}}$ (Fig. 5). The cerebrum showed a similar growth and decay pattern with an even slower discharge the $t_{\frac{1}{2}}$ of which could not be computed (Fig. 5).

Retained radioactivity in the whole body

Considering as 100% the amount of radioactivity initially injected, the amount retained in the whole body fell rapidly to 16% by the 23rd day (Fig. 6), the discharge being mainly through the faster component with $t_{\frac{1}{2}}$ of 6 days (Fig. 4). Then it fell gradually to 14% by the 30th day and to 5% by the 130th day, but 3% was still present on the 278th day.

Measurement of radioactivity of the stools was not a very reliable procedure, but on the whole a little under half of the injected radioactivity was excreted by the end of the 1st wk. This was consistent with the counts in the whole body.

Relative retention of radioactivity in organs

Figs. 7–9 show the relative retention of radioactivity in various organs. As in the case of specific activity the relative retention could again be considered by dividing the organs into three groups. The highest activity was retained in the initial stages of the experiment by the cellular and glandular organs mentioned earlier, even while the whole body showed a sharp fall (compare Figs. 6 and 7).

All the tissues of the central nervous system again, unlike the rest of the organs, showed increasing relative retention throughout the period of the experiment (Fig. 9). The cerebellum, the thalamus, and the lentiform nucleus exhibited maximum relative retention at all times.

While the three constituents of the carcass (bone, muscle, and skin) showed among the lowest values of relative retention (Fig. 8), their realtive retention did not fall with the passage of time and, in fact, rose in the case of muscle. Fig. 6 reveals this feature more strikingly.

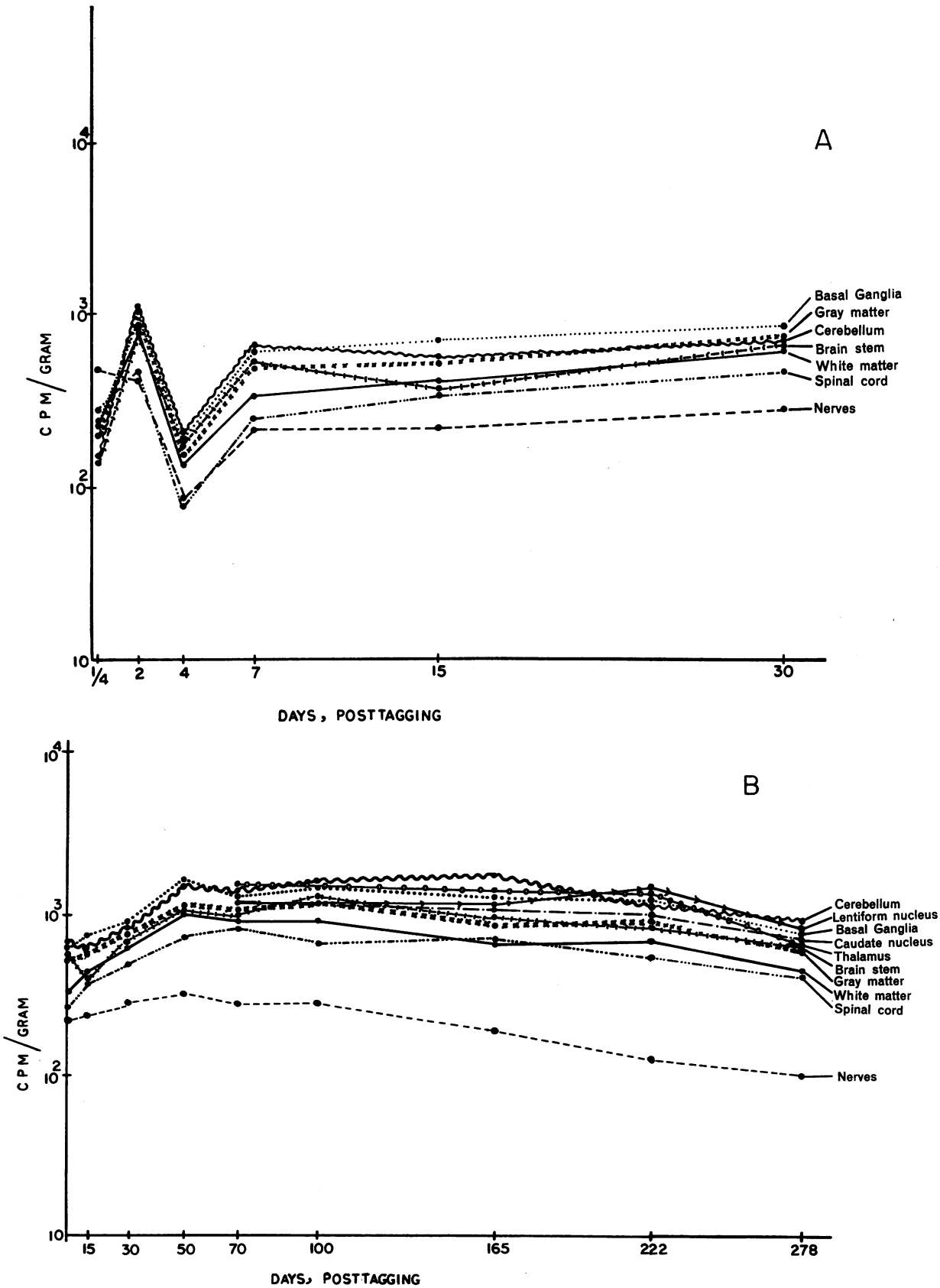
Retention of radioactivity in the carcass

The activity in the entire carcass is also plotted in Fig. 6 for 9 of the 12 animals where this procedure was carried out. It is pertinent to note the slope of the curve which rises steeply and linearly from the 6th hr to the 100th day and then flattens out into a plateau. Thus, the retained radioactivity in the carcass was seen to bear an inverse relationship to that in the whole body (compare the two graphs in Fig. 6). The carcass was found to constitute from 73 to 83% of the body weight of the monkey. Besides bone, muscle, and skin, the carcass also included most of the fibrous tissue in the body and many of the blood vessels and nerves, but these were not expected to affect significantly the pattern of discharge of ^{54}Mn from the carcass (see Fig. 9 for nerves).

DISCUSSION

The isotope used. $^{54}MnCl_2$ was preferred in the investigation reported here as its relatively long half-life of nearly 300 days permitted this long-term experiment for 278 days. This was considered essential so as to be able to extrapolate our findings, though of course guardedly and to a limited extent, to the human problem of chronic manganese toxicity.

Blood. Borg and Cotzias (11) found that after intravenous injection of ^{54}Mn in human beings 70% of the normal manganese left the circulation each minute and the remainder was excreted more slowly. Mahoney and Sargent (12) observed that 80% or more of the radioactivity in man disappeared from the plasma as a simple exponential with a $t_{\frac{1}{2}}$ of between $1\frac{1}{2}$ and 5 min, with the remainder declining at a much slower rate. In our observations of the blood in all animals from the 2nd day on, two peaks of activity were observed at around the 15th and 70th day, respectively, after the intraperitoneal injection. The high dilution of initial blood radioactivity implies that it acts as a rapid transport medium from the peritoneum to the various organs and tissues. The two periods of peak activity are obviously a reflection of discharge from the organs at those times and indicate the dynamic flux of manganese between blood and tissues.


A
C P M / GRAM
10^4
10^3
10^2
10
1/4
2
4
7
15
30
DAYS, POSTTAGGING
Basal Ganglia
Gray matter
Cerebellum
Brain stem
White matter
Spinal cord
Nerves
B
C P M / GRAM
10^4
10^3
10^2
10
15
30
50
70
100
165
222
278
DAYS, POSTTAGGING
Cerebellum
Lentiform nucleus
Basal Ganglia
Caudate nucleus
Thalamus
Brain stem
Gray matter
White matter
Spinal cord
Nerves

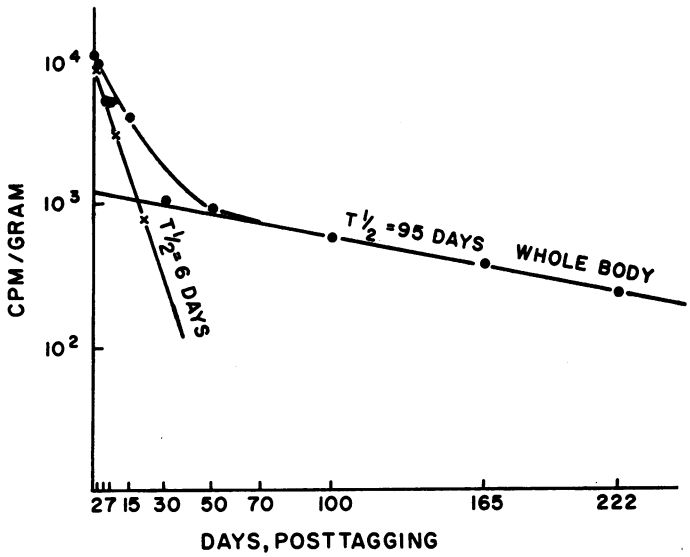


FIGURE 4 Exponential analysis of specific activity in the whole body. Note two clear components of discharge.

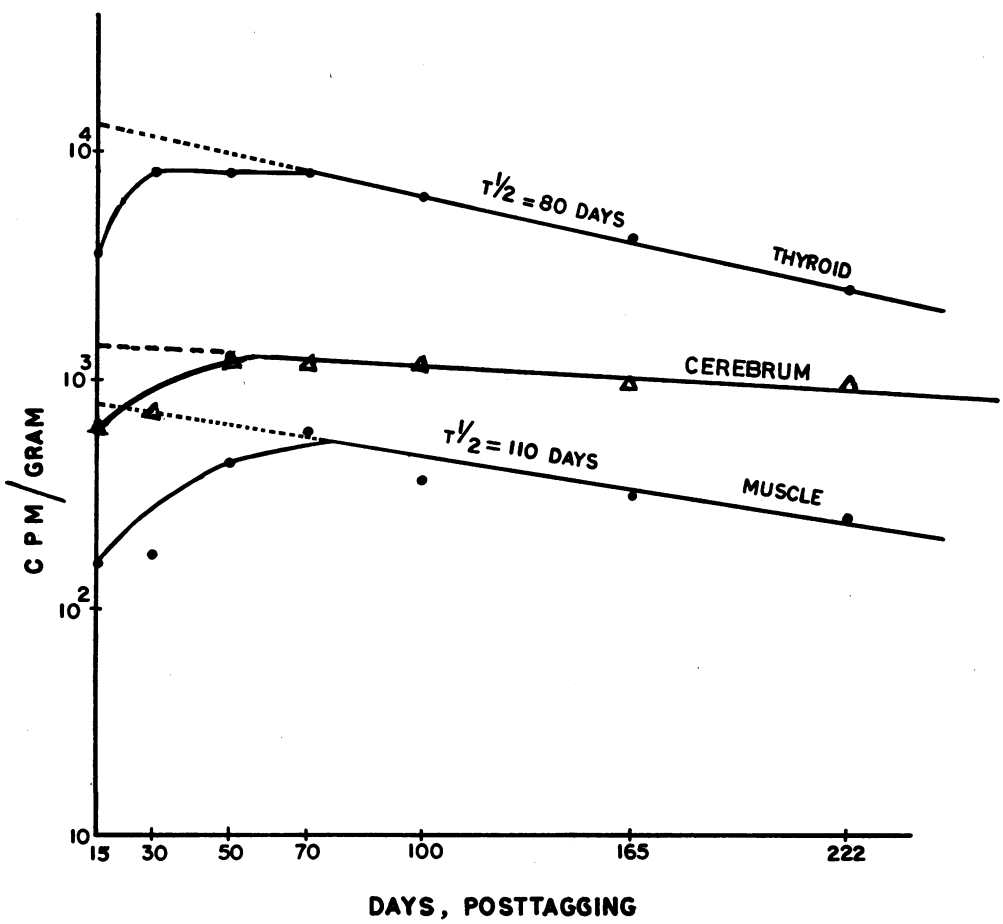


FIGURE 5 Exponential analysis of activity in the thyroid, muscle, and cerebrum showing prolonged $t\frac{1}{2}$ of one component for the former two, and an almost steady state of activity for the cerebrum (compare with Fig. 4).

FIGURE 3 (A and B) Specific activity, recorded and expressed as in Fig. 1, of the various components of the CNS showing the relatively greatest activity in the cerebellum and lentiform nucleus, the least in the cerebral white matter and the spinal cord, and much less in the nerves.

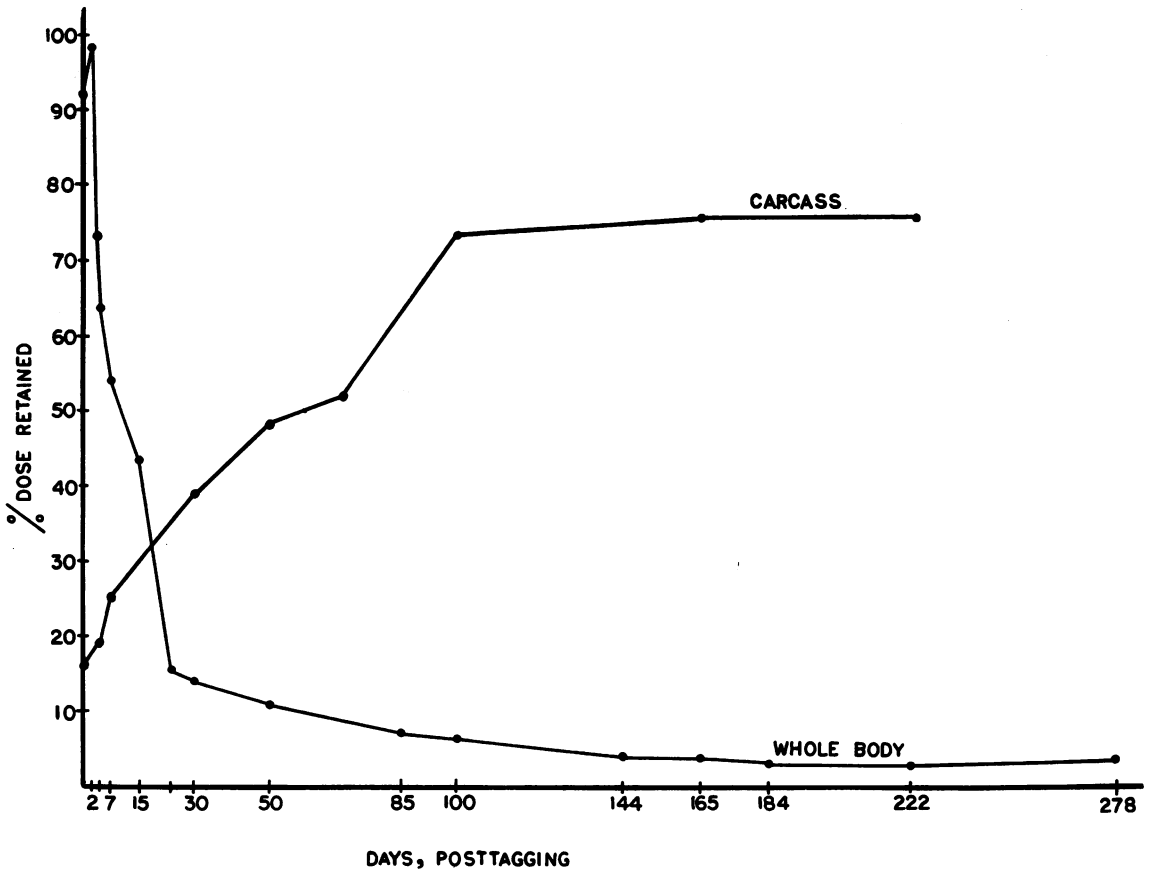


FIGURE 6 Percentage retention of injected radioactivity in the whole body and in the carcass for the full period of the experiment. Note the great reduction in the retained dose in the whole body by the 23rd day.

Mahoney and Small (13) found radiomanganese rapidly clearing from the plasma and reappearing irreversibly bound to red cells with a maximum radioactivity in the blood 10–20 days after the injection. We do not have separate observations on the red blood cells.

Individual organs. From these studies it is obvious that the most cellular organs, such as the endocrine glands, the liver, the kidney, and the constituents of the gastrointestinal system, showed the highest levels of specific activity, most of the viscera showed relatively less activity, the CNS still less, and the constituents of the carcass the lowest activity.

2 hr after tagging, radioactivity was located primarily in the cellular organs; whereas with passing time the CNS, endocrine glands, and to a lesser degree the carcass, especially the muscle, contained increasingly higher proportions of radiomanganese. Hence it appears that most viscera contained more readily dischargeable manganese than did the brain, the endocrines, or the carcass. While the carcass constituted 73–83% of the body weight of the whole monkey, it contained 17–53% of the retained activity in the whole body for a period up to 70 days. After the 100th day, the retained activity in the carcass was 75% of the dose retained in the whole body. Britton and Cotzias (14) also found that in mice injected with ^{54}Mn the radioactivity retained in the carcass ranged between 78 and 93% of the total. The falling trend of retained activity in the whole body could be a reflection of the trend in most organs but not in the constituents of the carcass or the CNS.

While the endocrine glands were not of direct interest to us in our present studies, it is interesting to note not only the very high specific activity of the suprarenal, thyroid, and pituitary glands, but also the very high relative retention of ^{54}Mn by these three glands. A comparison of Fig. 7 with Figs. 8 and 9 shows that even in the later stages of the experiment the relative retention in these glands is over 1.5 times as high as that in the CNS. The thyroid is particularly interesting in this respect, since it has been reported earlier (15) that man-

ganese, an element with an atomic structure resembling that of the halogens, is found in greater amounts in the thyroid than in other organs. Reference to the Periodic Table of Elements reveals that halogens and manganese and technetium are in the same group, i.e., No. VII. Tc^{99m} is now widely used in the scanning of thyroid and brain along with other organs (16).

All tissues in the monkey exhibited two peaks of activity at about the 7th and the 70th day corresponding to the 4th and the 13th day in the rat (8), which suggests that there are two different pools or phases of activity. The curve for many organs could be resolved roughly into two exponential components, one slow and one fast, with varying half-times for different organs. However, for the CNS, thyroid, and muscle, only one discharge component could be calculated because there was a steady state of retention of manganese.

The brain appears to be peculiar in that it contains relatively little manganese normally (18), but it has the property of retaining it for relatively long periods (up to 278 days) in amounts out of all proportion to that retained by other organs. This confirms our earlier finding in the rat when the period of observation was 64 days (9). We have been unable to find information on any other heavy metal being selectively retained in the brain. Walshe has never found any evidence for radiocopper, either ^{67}Cu or ^{64}Cu, in the brain in patients with Wilson's disease up to 2 wk after intravenous injection.[2]

All the components of the nervous system were found to retain rather than to discharge the injected ^{54}Mn, the nerves exhibiting the least radioactivity and the lentiform nucleus and the cerebellum showing relatively the highest values at all times. To the best of our knowledge, there has been no report of observations on the monkey with any isotope of manganese, which is designed to investigate localization of manganese in different parts of the CNS. In fact, as evidenced in our first paper, even when other isotopes have been used, the important question of selective vulnerability

[2] Walshe, J. M. Personal communication to D. K. Dastur.

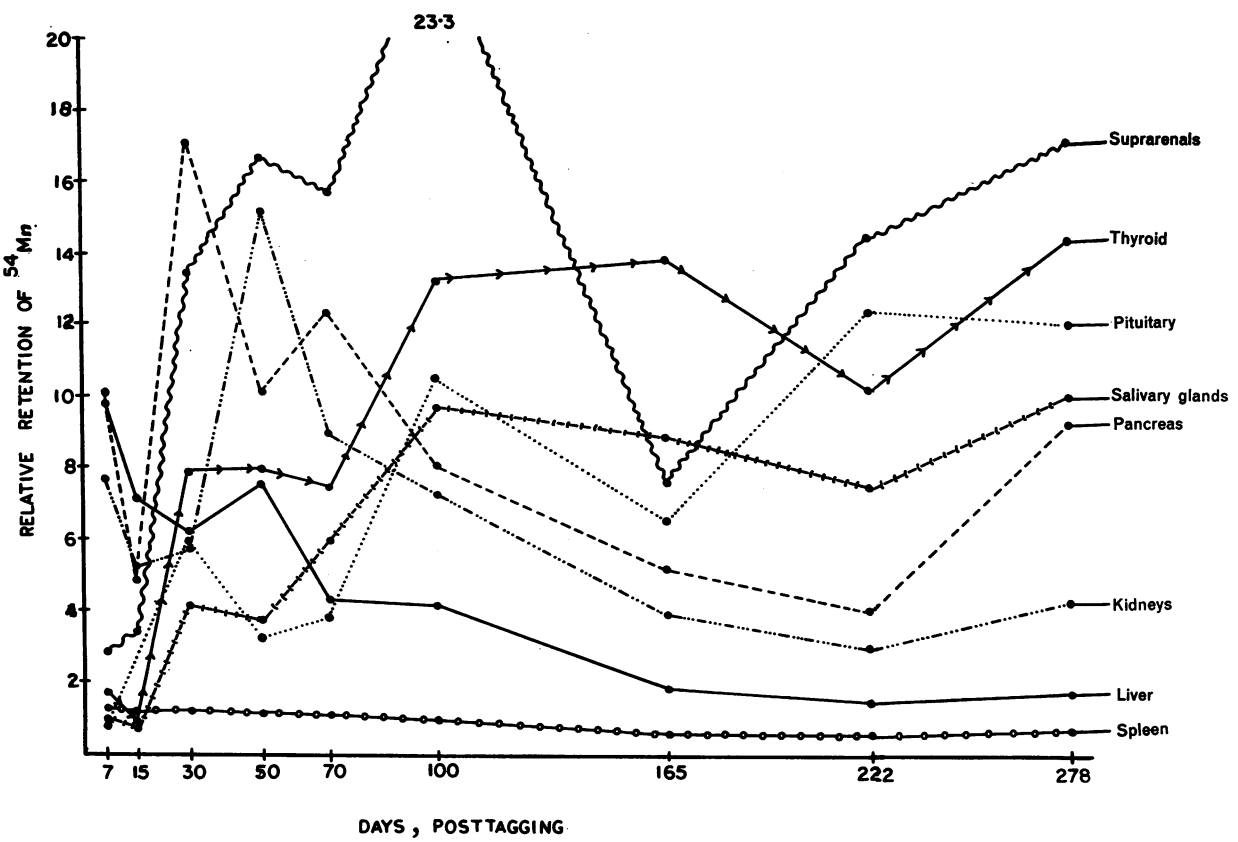


FIGURE 7 Relative retention of ^{54}Mn from the 7th to the 278th day in the more cellular organs, with the highest activity in the endocrine glands and the least in the spleen.

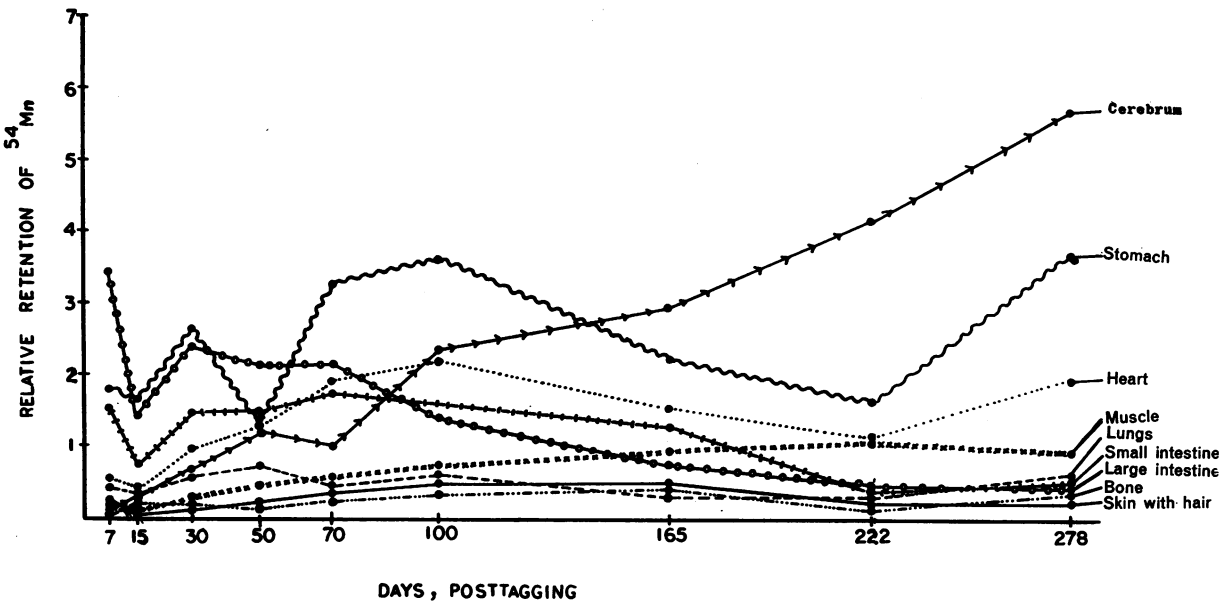


FIGURE 8 Relative retention, expressed similarly to that in Fig. 7, in other organs. Note the increasing relative retention in the cerebrum and the relatively low retention in the components of the carcass.

of the brain in chronic manganism has not been approached directly.

Relationship of our experimental observations to human manganism. In the present experiment gradual uptake of isotopic manganese by the brain continued until about the 50th day; and from then till the 278th day, there was persistent saturation with increase in relative retention. As suggested for the rat (8, 9), this might be the result of a basic inability on the part of the CNS to discharge manganese. That in turn might be responsible for its selective vulnerability in chronic manganism in man, where large amounts of manganese dust are inhaled and a predominantly neurological disorder develops. The relatively greater specific activity and retention of ^{54}Mn observed by us in the basal ganglia seems pertinent from the point of view of the clinical picture in manganese miners, a conspicuous feature of which is a parkinsonian basal ganglionic disorder (3). Damage to any of these areas in experimental animals is known to produce characteristic neurological pictures of hyperkinesia, tremor, rigidity, etc. (18).

Borg and Cotzias (11), Mena, Fuenzalida, and Cotzias (19), and Cotzias, Koriuchi, Fuenzalida, and Mena (20) probed several regions of the body and carried out whole-body counting of ^{54}Mn in miners with neurotoxicity, healthy miners, and normal subjects. They found the highest activity in healthy miners and concluded that neurotoxicity was not dependent on high levels of manganese and hence chelating agents would not be useful (20). Penalver (21) observed some improvement in a proportion of his cases after administration of ethylenediaminetetraacetic acid. Moreover, since the methods used by Cotzias et al. (20) on human patients and healthy subjects could not include direct estimation of radioactivity in various body organs and parts of the brain as our study did, we feel that a selectively high retention of ^{54}Mn in the CNS could well be the basis of damage to the brain. The possibility thus remains (7) that the constant perfusion of the neurons with high levels of circulating manganese in the blood may lead to an irreversible and deleterious chemical change especially in intracellular micelles such as the mitochondria. It is of some relevance to mention here that the differential centrifugation of homogenates of the liver, white matter, grey matter, and the basal ganglia in the last two of our monkeys showed maxmium activity in the mitochondrial fraction and much less activity in the nuclear fraction and the supernate, respectively.

An alternate or additional mechanism for the selective damage to the brain might be the difference in rates of turnover of manganese in different organs, as suggested by Kato (22) and as, in fact, we have observed in our experiments on both the rat and the monkey.

Finally, one biophysical feature of relevance to the selective retention of manganese in the brain may be mentioned. Most metals have low electrical resistivity.

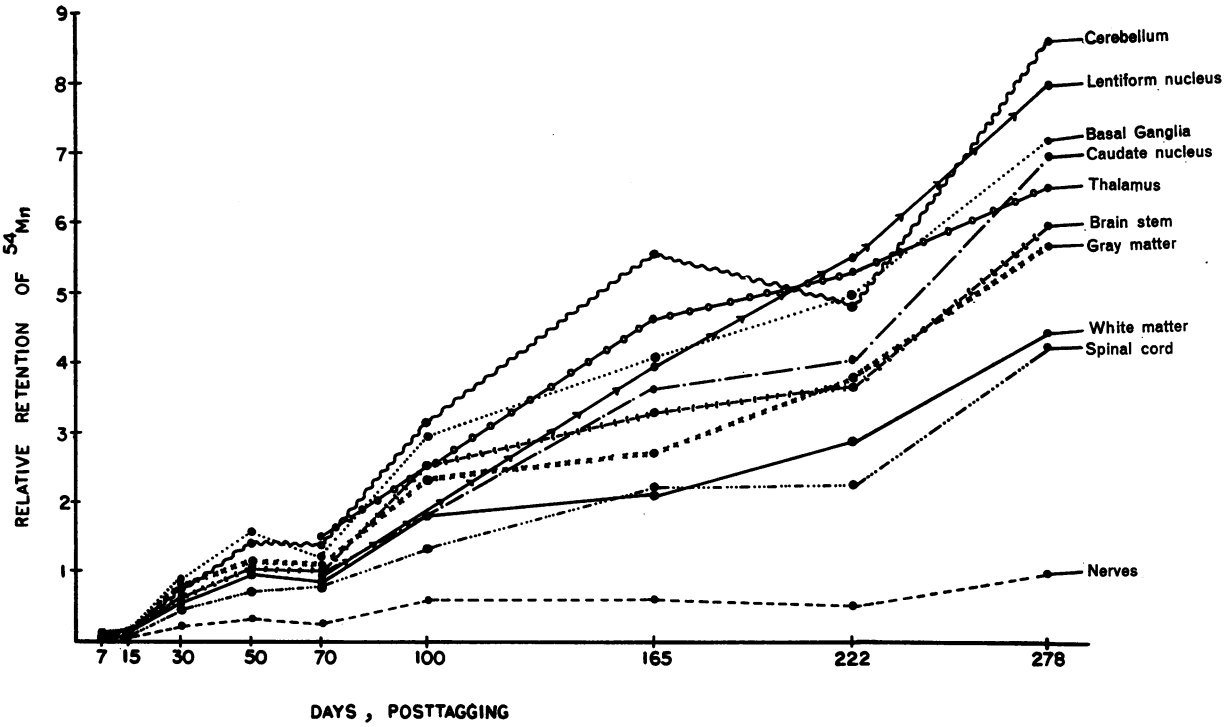


FIGURE 9 Relative retention in the various components of the CNS with the cerebellum and lentiform nucleus showing the most and the cerebral white matter and spinal cord the least; the nerves retain only insignificant amounts.

The resistance of copper, for example, is only 10 microhm-cm even at 1000°C. Among the metals, manganese is reported to have the greatest electrical resistivity. At room temperature it is about 188 microhm-cm (23). Whether this high electrical resistivity of manganese is of any significance in the production of brain damage in chronic manganism is unknown at the present time but may be a subject of interesting study.

ACKNOWLEDGMENTS

Thanks are due to Dr. R. D. Ganatra, officer-in-charge at the Radiation Medicine Centre, for facilities provided.

REFERENCES

1. Manganese Poisoning Enquiry Committee Report. 1960. Ministry of Labour and Employment. Government of India. 143.
2. Penalver, R. 1955. Manganese poisoning. *Ind. Med.* **24**: 1.
3. Canavan, M. M., S. Cobb, and C. K. Drinker. 1934. Chronic manganese poisoning, report of a case with autopsy. *Arch. Neurol. Psychiat.* **32**: 501.
4. Stadler, H. 1935. Zur histopathologie des gehirns bei manganvergiftung. *Z. Gesamte Neurol. Psychiat.* **154**: 62.
5. Van Bogaert, L., and M. J. Dallemagne. 1945. Approaches experimentales des troubles nervaux du manganisme. *Monatsschr. Psychiat. Neurol.* **111**: 60.
6. Pentschew, A., F. F. Ebner, and R. M. Kovatch. 1963. Experimental manganese encephalopathy in monkeys. *J. Neuropathol. Exp. Neurol.* **22**: 488.
7. Cotzias, C. C. 1958. Manganese in health and disease. *Physiol. Rev.* **38**: 503.
8. Dastur, D. K., D. K. Manghani, K. V. Raghavendran, and K. N. Jeejeebhoy. 1969. Distribution and fate of Mn^{54} in the rat, with special reference to the CNS. *Quart. J. Exp. Physiol. Cog. Med. Sci.* **54**: 322.
9. Manghani, D. K., D. K. Dastur, K. N. Jeejeebhoy, and K. V. Raghavendran. 1970. Effect of stable manganese on the fate of radiomanganese in the rat with special reference to the CNS. *Indian J. Med. Res.* **58**: 209.
10. Manghani, D. K. 1969. Distribution and turnover of isotopic manganese in the rat and the monkey: with special reference to the CNS. Doctorate Thesis. University of Bombay.
11. Borg, D. C., and G. C. Cotzias. 1958. Manganese metabolism in man. Rapid exchange of Mn^{56} with tissue as demonstrated by blood clearance and liver uptake. *J. Clin. Invest.* **37**: 1269.
12. Mahoney, J. P., and K. Sargent. 1967. The plasma disappearance and erythrocyte uptake of Mn^{54}. *J. Clin. Invest.* **46**: 1090.
13. Mahoney, J. P., and W. J. Small. 1968. Studies on manganese. III. The biological half-life of radiomanganese in man and factors which affect this half-life. *J. Clin. Invest.* **47**: 643.
14. Britton, A. A., and G. C. Cotzias. 1966. Dependence of manganese turnover on intake. *Amer. J. Physiol.* **211**: 203.

15. Ray, T. W., and L. J. Deysach. 1942. Storage of manganese by thyroid. Effect on oxygen consumption of the guinea pig. *Proc. Soc. Exp. Biol. Med.* **51**: 228.
16. Silver, S. 1968. Studies of the central nervous system including studies of cerebral blood flow. *In* Radioactive Nuclides in Medicine and Biology. E. H. Quimby, editor. Lea and Febiger, Philadelphia, Pa. 3rd edition. Chap. 16. 424
17. Hanig, R. C., and M. H. Aprison. 1967. Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc and chloride concentrations in several brain areas. *Anal. Biochem.* **21**: 169.
18. Denny-Brown, D. 1962. The Basal Ganglia and Their Relation to Disorders of Movement. The Oxford University Press, London.
19. Mena, I., O. Marin, S. Fuenzalida, and G. C. Cotzias. 1967. Chronic manganese poisoning. Clinical picture and manganese turnover. *Neurology.* **17**: 128.
20. Cotzias, G. C., K. Koriuchi, S. Fuenzalida, and I. Mena. 1968. Chronic manganese poisoning—clearance of tissue manganese concentration with persistence of the neurological picture. *Neurology.* **18**: 376.
21. Penalver, R. 1957. Diagnosis and treatment of manganese intoxication, report of a case. *A.M.A. Arch. Ind. Health.* **16**: 64.
22. Kato, M. 1963. Distribution and excretion of radiomanganese administration to the mouse. *Quart. J. Exp. Physiol. Cog. Med. Sci.* **48**: 355.
23. Miner, W. N. 1968. Plutonium. Division of technical information, series on "understanding the atom." U. S. Atomic Energy Commission, Washington, D. C. 26.