The Distribution of ^{65}Zn in Mice Brain

I. Topographical Peculiarity of the Distribution of ^{65}Zn in the Brain of Intact Mice

Masataka FUJII
2nd Division, Department of Surgery
(Director: Prof. Shunji Tokuoka)
Yamaguchi University School of Medicine
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Introduction

In 1955, Maske[18] found that a part of the Ammon's horn of mammals was stained by supravital stain of dithizone (diphenylthiocarbazone) and supposed that this reaction indicated existence of zinc. The same observation was determined by Fleischhauer & Horstmann (1957),[7] Timm (1958)[33] and Otsuka & Umetani (1962).[28]

Although these histochemical reactions are not specific for zinc, Klee & Liefländer (1965)[16] showed higher zinc content in the Ammon's horn in rabbits. On the other hand, von Euler (1961)[4] and Otsuka & Kawamoto (1966)[27] showed the uptake of ^{65}Zn in the Ammon's formation (Ammon's horn & dentate gyrus) from their autoradiographic observation.

Although the above investigations suggest an intimate relationship of zinc to the Ammon's formation, the significance of zinc in the central nervous system is scarcely explained.

This experiment is undertaken in an attempt to investigate the peculiarity of zinc distribution in the brain by means of ^{65}Zn. For this purpose, the brain was divided into five parts and the time course of their ^{65}Zn activity was compared with each other. On the other hand, autoradiographic study was performed to examine the localization of ^{65}Zn in the brain.

Material and Methods

Male dd strain mice (about 20g in weight) were generally used in the present investigation. The animals were maintained on Oriental Chow with free access to food and water. Radioactive zinc was obtained from New England Nuclear Corp. (U.S.A.) as $^{65}ZnCl_2$ solution.

Each mouse was injected subcutaneously a single dose of about 4μc of $^{65}ZnCl_2$. Reagents were dissolved in physiological saline solution. The injection volume was 0.1 ml. The animals were sacrificed by decapitation at various intervals up to 720 hours. The brain was dissected into the following parts according to the schema of Ogawa[25]: 1) cerebrum 2) Ammon's formation (hippocampus & dentate gyrus) 3) diencephalon 4) mesencephalon, pons & medulla oblongata and 5) cerebellum.

Each sample was not perfused since ^{65}Zn activity in blood was negligible after the 18 hour interval. The tissues were dissolved into conc. nitric acid and analysed for ^{65}Zn activity in a well-type gamma-scintillation counter. No radioactive decay correction was made.

For determining water content of various parts of the brain, the samples were dried in electric oven at 110°C for 12 hours.

Autoradiographic study: intracerebral injection was performed by our modification[8] of the method of Halley and McCormic (1957).[12] Preliminary experiments using Evans' blue showed that most of the dye injected intracerebrally was distributed in the ventricular spaces. The animals were injected a single dose of about 1 μc of $^{65}ZnCl_2$. Reagents were dissolved in Ringer's solution. The injection volume was 0.01 ml. The animals were decapitated at various intervals up to 240 hours. The brain was fixed in 100 % alcohol solution and was cut by paraffin section. These sections were managed with "stripping film" (Fuji ET-2E) according to inverting method and then developed after being exposed for 30 days at about 4°C in dark room.[17]

Results

The ^{65}Zn activity in five parts of the brain at different intervals up to 720 hours after subcutaneous injection is shown in Table 1 and Fig. 1.

Table 1. ^{65}Zn activity in various parts of the brain at different times after subcutaneous injection. Each value is expressed as per cent per mg w.w. of injected dose and is the average of six mice.

	Hours after injection				
	24	48	120	240	720
Cerebrum	1.54 ± 0.22	1.72 ± 0.36	1.92 ± 0.23	1.37 ± 0.12	0.43 ± 0.07
Ammon's formation	1.54 ± 0.19	1.77 ± 0.35	1.98 ± 0.30	1.59 ± 0.43	0.74 ± 0.17
Diencephalon	1.62 ± 0 16	1.66 ± 0.43	1.65 ± 0.22	1.05 ± 0.35	0.33 ± 0.04
Mesencephalon, Pons & Medulla oblongata	1.72 ± 0.22	1.86 ± 0.40	1.80 ± 0.25	1.00 ± 0.09	0.36 ± 0.11
Cerebellum	1.73 ± 0.24	1.94 ± 0.61	1.83 ± 0.21	1.32 ± 0.09	0.43 ± 0.04
Whole brain	1.61 ± 0.19	1.76 ± 0.38	1.80 ± 0.24	1.28 ± 0.09	0.42 ± 0.07

average ± S.D.

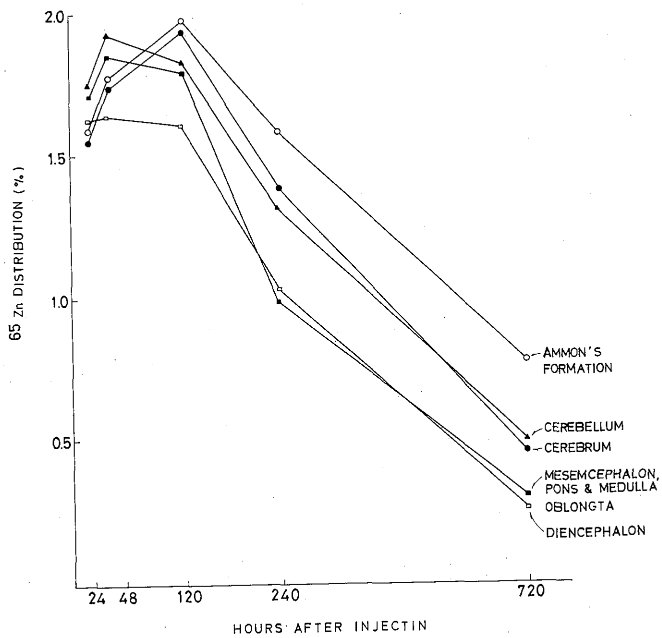

Fig. 1. ^{65}Zn activity in various parts of the brain at different times after subcutaneous injection.

The ^{65}Zn activity of whole brain increased gradually and reached its maximum of about 1.8 % at the 120 hour interval. In the cerebellum, diencephalon and mesencephalon, pons & medulla oblongata, the ^{65}Zn activity reached its maximum at the early interval (the 48 hour interval). In the Ammon's formation and cerebrum, however, it showed maximal deposition at the 120 hour interval. In these intervals, however, there was no significant difference in the ^{65}Zn activity of each part of the brain. The rate, at which ^{65}Zn decreased in the Ammon's formation, was slower than that in other parts and the ^{65}Zn activity of this part was about twice as high as in the remaining parts.

In order to determine these difference in each part more definitely, relative activity was calculated; the values were expressed as per cent of (^{65}Zn activity in each part/mg w.w.) / (^{65}Zn activity in whole brain/mg w.w.). Although the relative activity of each part was practically the same at the 48 hour interval, as shown in Table 2 and Fig. 2, the different parts of the brain were divided into three groups according to their relative activity at the late intervals (the 240 and 720 hour intervals).

The first group which showed a lower activity consisted of the diencephalon and mesencephalon, pons & medulla oblongata, while the second group comprised the cerebrum and cerebellum showed a higher one. The highest ^{65}Zn activity of the Ammon's formation was the most remarkable finding. The activity of this part was about twice as high as in the diencephalon at the 720 hour interval.

Table 2. Relative ^{65}Zn activity in various parts of the brain at different times after subcutaneous injection.
The activity is given as the following ratio: (^{65}Zn content in each part / mg w.w.) / (^{65}Zn content in whole brain / mg w.w.) $\times$ 100 %.
Each value is the average of six mice.

	Hours after injection				
	24	48	120	240	720
Cerebrum	96 ± 4	98 ± 3	107 ± 18	107 ± 3	102 ± 5
Ammon's formation	94 ± 6	102 ± 10	112 ± 4	123 ± 23	176 ± 22
Diencephalon	101 ± 4	94 ± 8	81 ± 7	82 ± 5	80 ± 12
Mesencephalon, Pons & Medulla oblongata	107 ± 14	1C6 ± 9	100 ± 8	77 ± 7	83 ± 15
Cerebellum	107 ± 8	1C9 ± 20	105 ± 13	102 ± 11	1C4 ± 9

average ± S.D.

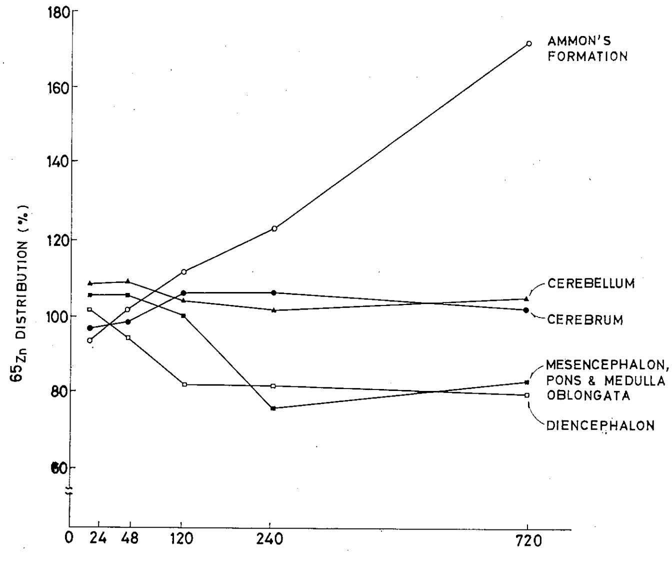

Fig. 2. Relative ^{65}Zn activity in various parts of the brain at different times after subcutaneous injection. The activity is expressed as per cent of ^{65}Zn activity in each part / mg w.w. / ^{65}Zn activity in whole brain/mg w.w.

Water content in five parts of the brain is shown in Table 3. Since there was no correlation between the ^{65}Zn activity and the water content of each part, the difference of ^{65}Zn activity was not due to the difference of the water content.

Table 3. Water content of various parts of the brain.
Each value is expressed as per cent of wet weight.

Cerebrum	Ammon's formation	Diencephalon	Mesencephalon, Pons & Medulla Oblongata	Cerebellum
79.1 ± 0.7	79.8 ± 2.0	79.7 ± 2.0	76.2 ± 0.8	77.4 ± 0.9

Autoradiographic observation.

In case of subcutaneous injection, the ^{65}Zn activity in the brain was only about 2 per cent per g. Therefore it would not be surprising to find that a single dose of 50 μc of $^{65}ZnCl_2$ did not show satisfactorily high concentration autoradiographically. From the above observation, intracerebral injection technique was chosen in the present study. The localization of the grain in the Ammon's formation was not found at the 24 and 48 hour intervals, but low density of the grain was diffusely found in all areas of the brain. The most characteristic finding was obtained at the 120 hour interval. As shown in Fig. 3,

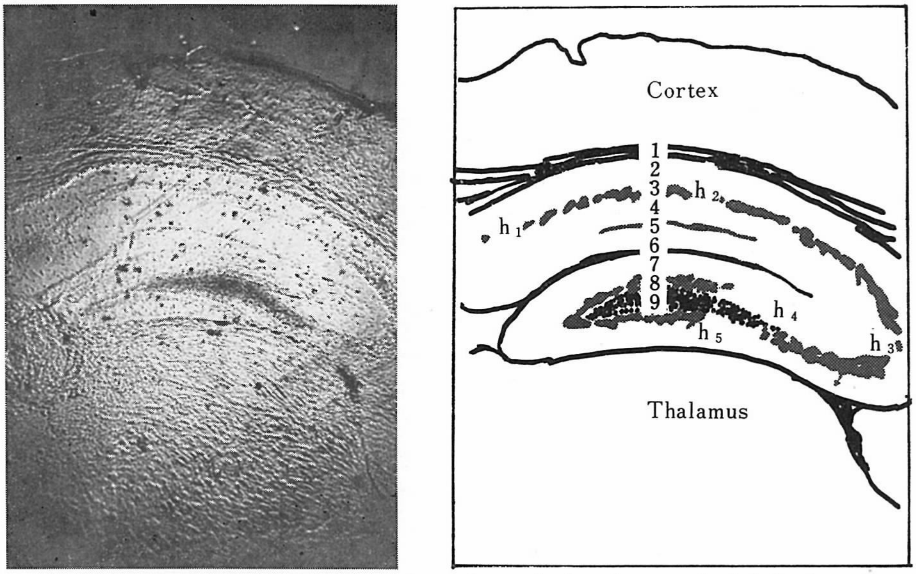

Fig. 3. Autoradiography at the 120 hour interval after intracerebral injection of ^{65}Zn. The grain is observed in the marginal part of stratum radiatum of h_4, h_5 area which is contact with stratum pyramidale of the hippocampus and stratum multiforme of dentate gyrus.

Hippocampus:
1. Alveus
2. Stratum multiforme
3. Stratum pyramidale
4. Stratum radiatum
5. Stratum lacunosum
6. Stratum moleculare

Gyrus dentatus:
7. Stratum moleculare
8. Stratum granulosum
9. Stratum multiforme

the grain of ^{65}Zn was localized in the Ammon's formation; according to the classification of Rose,[30] high density of the grain was observed in the marginal part of stratum radiatum of h_4,h_5 area which is contact with stratum pyramidale of the hipppocampus and stratum multiforme of the dentate gyrus. Except for h_3 area, these findings were consistent with the histochemical observation.

At the 240 hour interval, almost all grain of ^{65}Zn was removed from the brain autoradiographically. This finding is consistent with the rapid decrease of subcutaneously injected ^{65}Zn after 120 hours.

Discussion

The present study showed that ^{65}Zn activity was different in various parts of the brain. Especially, the relative ^{65}Zn activity, represented as the ratio of its incorporation in each part to whole brain, showed the characteristic distribution of ^{65}Zn in the brain.

Since there was no significant difference in ^{65}Zn activity at the early intervals, it is unlikely that the difference in ^{65}Zn distribution is due to that in its entry into each part. This is supported by the autoradiographic finding that intracerebrally injected ^{65}Zn was localized in the Ammon's formation, in contrast with the lack of the localization in other parts. In this technique, participation of the blood-brain barrier can be excluded.

Distribution of ^{65}Zn in the brain at the late intervals is nearly consistent with that of zinc content in rabbits reported by Klee & Liefländer (1965),[16] except for the mesencephalon, pons & medulla oblongata.

It is noteworthy that ^{65}Zn activity was highest in the Ammon's formation, indicating the special affinity of ^{65}Zn for this part.

With respect to zinc in the Ammon's formation, it has been shown that dithizone reaction or Timm's reaction (sulfate-silver method) is positive in this part.[7][18][28][33] Unfortunately these reactions are not specific for zinc. The present autoradiographic study demonstrated that the localization of ^{65}Zn in the Ammon's formation was consistent with the histochemical localization of zinc, with a minor difference that the grain of ^{65}Zn was not found autoradiographically in stratum radiatum of h_3 area. This correspondence clearly indicates that zinc has a characteristic distribution in the Ammon's formation.

Otsuka & Kawamoto (1966) [27] observed autoradiographically that, after intraperitoneal injection of a single dose of 50 μc of ^{65}Zn-gluconate, ^{65}Zn was uptaken in the Ammon's formation within 13-24 hours, retained there for 1-2 days and then diminished after 2-5 days. From the above observation, they concluded that ^{65}Zn in this part turned over as rapid as 1 to 2 days. In the present study, however, the relative ^{65}Zn activity in this part increased as time lapsed, although the absolute activity decreased rapidly after 5 days. This observation indicates that loss of ^{65}Zn in the Ammon's formation is slower than that of other parts.

High activity of various enzymes and high metabolic rate of RNA in the Ammon's formation[9][26][28] suggest active metabolism in this part. Therefore, prolonged retention of ^{65}Zn may have some connection with the peculiarity of the metabolism in the Ammon's formation.

The significance of zinc in the Ammon's formation is scarcely understood. Since almost all of ^{65}Zn in the brain is thought to be combined with protein,[35] it is resonable to presume that zinc exists as zinc metalloprotein, in which metal is combined with protein in a unique manner so that two can be entity in nature, or as zinc-protein complex in which metal combined reversely.

From the histochemical observation, Fleischhauer (1958),[6] Ortmann (1961)[26] and Mc Lardy (1962)[19] considered that zinc in the Ammon's formation existed as a constituted element of carbonic anhydrase or acid phosphatase. Keller & Peters (1959)[15] and Klee & Liefländer (1965),[16] however, showed that the activity of carbonic anhydrase was not affected by zinc complex-forming agents such as dithizone and ethylenediamino-tetraacetic-acid. Therefore, it is unlikly that zinc metalloprotein reacts with dithizone or H_2S histochemically.

Moreover, Tupper et al. (1952)[36] observed that carbonic anhydrase kept as long as 30 days in solution of ^{65}Zn-containing zinc salt took up no significant amount of ^{65}Zn. Therefore, the present observation that ^{65}Zn, as the chloride, concentrated comparatively early in the specific layer of the Ammon's formation seems to deny that ^{65}Zn entered into the composition of enzymes such as carbonic anhydrase.

Summary

Peculiarity of zinc distribution in mice brain was studied by means of ^{65}Zn. For this purpose, the ^{65}Zn activity of five parts of the brain was investigated at different interval up to 720 hours after subcutaneous injection and autoradiography of the brain was examined after intracerebral injection of ^{65}Zn.

The results obtained were as follows:

1) The ^{65}Zn activity in each part showed its maximal deposition at the 48 to 120 hour interval. But the activity did not show significant difference one another.
2) The ^{65}Zn activity in each part decreased after the 120 hour interval. The rate at which ^{65}Zn decreased in the Ammon's formation was slower than that in other parts.
3) At the late intervals, five parts of the brain were divided into three groups according to their ^{65}Zn activity. The diencephalon and mesencephalon, pons & medulla oblongata showed lower activity, while the activity of the cerebrum

and cerebellum was higher. The Ammon's formation showed the highest ^{65}Zn activity.

4) In autoradiographic study, the high density of ^{65}Zn grain was observed in the marginal part of stratum radiatum of h_4,h_5 area which is contact with stratum pyramidale of the hippocampus and stratum multiforme of the dentate gyrus.

(References are presented in part II.)