The Distribution of ^{65}Zn in Mice Brain

II. The Effect of Subtotal Pancreatectomy on ^{65}Zn Distribution in Tissues, with Special Reference to Changes in the Brain

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Introduction

In 1951, Tokuoka[34] reported that the caudal resection of approximately two third of the pancreas was effective in alleviating the epileptic seizures in 66.7 % of 27 cases of genuine epilepsy, the results being better in childhood. On the basis of this observation, he suggested that the disturbance of zinc metabolism might play an important role in the neurochemical background of the alleviating effect.

With respect to the relationship of zinc to brain excitability, Nagasue (1957)[23][24] and Hiraoka (1966)[14] have shown that zinc deficiency decreases brain excitability while zinc intoxication increases it. Moreover, it has been shown that zinc is predominantly detectable in the Ammon's formation,[6][7][18][19][20][28][33] which is thought to be an important agency of epileptic seizures.[10][11] These observations indicate that zinc metabolism in the brain may have some effect on brain excitability.

On the other hand, it has been shown that the pancreas contains high average of zinc and is an excretory organ of zinc.[22][37] Although it is predictable that pancreatectomy causes the disturbance of zinc metabolism, literature dealing with the effect of subtotal pancreatectomy on zinc metabolism is lacking.

This study is undertaken in an attempt to investigate the effect of subtotal pancreatectomy on zinc distribution in tissues by means of radioactive zinc (^{65}Zn), especially to show what changes occur in ^{65}Zn distribution in various parts of the brain.

Material and Methods

Male dd strain mice (about 20g in weight) were used in the present study. The animals were maintained on Oriental Chow and tap water.

Radioactive zinc (^{65}Zn) was obtained from New England Nuclear Corporation. Subtotal pancreatectomy was performed as reported by Takashima.[32] The experimental animals were anesthetized with ether. After median laparotomy, the stomach was retracted to expose the mobil pancreas and the distal two third of the pancreas was removed. In the control mice the pancreas was only manipulated. The pancreatectomized and control mice were pair-fed. The experiment was carried out I week after the pancreatectomy. One group of animals were subcutaneously injected a single dose of 6 μc of $^{65}ZnCl_2$, dissolved in physiological saline solution. The injection volume was 0.15 ml. The animals were sacrificed by rapid decapitation at various intervals up to 720 hours. The blood was collected on a clean glass plate from the severed carotid artery. Then the liver and brain were removed. The brain was divided into five parts as described in part I. Another group was recieved a single dose of about 4 μc of $^{65}ZnCl_2$ by a silicone tube which was put into the stomach. In this case, $^{65}ZnCl_2$ was dissolved in drinking water. The administered volume was 0.2 ml. The animals were sacrificed at various intervals up to 240 hours. The samples were collected as described above, but the brain was not divided. For determining the body retention of ^{65}Zn, each carcass was dissolved in conc. sulfuric acid after separation of the content of the gastro-intestinal tract. The samples were treated as described in part 1 and analyzed for ^{65}Zn activity in a well-type gamma scintillation counter. For determining water content of the brain, the samples were dried in electric oven at 110°C for 12 hours.

Results

The distribution of subcutaneously injected ^{65}Zn in the brain, blood and liver at various intervals up to 720 hours is shown in Table 1. The ^{65}Zn activity in the brain showed a significant decrease at the 48 hour interval when it reached maximum deposition in the present study. The average value for the pancreatectomized and control mice was 1.6 % and 2.3 % respectively, and the difference was statistically significant at 5 % level. However, there was no significant difference between two groups at other intervals.

The ^{65}Zn activity in the blood and liver reached its maximum deposition within 3 hours and then decreased rapidly. There was no difference in ^{65}Zn activity in the liver between two groups, while the removal of ^{65}Zn from the blood was significantly delayed in the pancreatectomized mice at the 6 hour interval; the ^{65}Zn activity of the pancreatectomized mice was 2.68 % and was about twice higher than that of the control. However, it fell below the control value after 18 hours.

The time course of ^{65}Zn distribution following gastric gavage is shown in Table 2. In the pancreatectomized and control mice, the body retention of ^{65}Zn increased gradually, reached its maximum at the 24 hour interval, and then decreased gradually. There was no significant difference between two groups within 24 hours. However, the body retention of ^{65}Zn in the pancreatectomized

Table 1. Distribution of subcutaneously injected $^{65}ZnCl_2$ in the brain, blood and liver of subtotally pancreatectomized mice.

Hours after injection		3	6	18	48	240	720
Brain	Pancrea-tectomized	0.60±0.21 (6)	0.99±0.28 (6)	1.23±0.61 (6)	1.65±0.60* (7)	1.31±0.60 (6)	0.42±0.10 (5)
	Control	0.68±0.34 (6)	0·94±0.64 (6)	1.34±0.33 (6)	2.28±1.20 (6)	1.30±0.52 (6)	0.34±0.12 (5)
Blood	Pancrea-tectomized	4.85±3.18 (6)	2.68±0.81** (6)	1.70±0.32 (5)	0.85±0.15 (5)	0.55±0.22 (5)	under 0.1
	Control	4.96±0.95 (6)	1.21±0.37 (6)	1.19±0.28 (5)	1.07±0.21 (5)	0.72±0.15 (7)	
Liver	Pancrea-tectomized	24.0±3.1 (5)	22.8±4.3 (6)	15.2±3.5 (7)	12.1±2.0 (8)	4.0±1.1 (7)	0.4±0.1 (6)
	Control	22.6±7.7 (6)	19.9±3.6 (6)	18.1±2.0 (6)	12.8±1.8 (8)	3.3±1.0 (5)	0.4±0.1 (5)

Each value is expressed as per cent of injection dose per gram tissue (average ± S.D.).
( ): No. of mice * $P<0.05$ ** $P<0.01$

Table 2. ^{65}Zn activity following gastric gavage in subtotally pancreatectomized mice.

Hours after administration		12	24	48	240
Brain	Pancreatectomized	0.07 ± 0.03 (9)	0.12 ± 0.07 (7)	0.14 ± 0.03* (7)	0.13 ± 0.03 (6)
	Control	0.05 ± 0.01 (8)	0.13 ± 0.07 (9)	0.21 ± 0.06 (7)	0.18 ± 0.11 (6)
Blood	Pancreatectomized	0.08 ± 0.02 (8)	0.08 ± 0.05 (6)	0.05 ± 0.02 (7)	0.03 ± 0.01 (5)
	Control	0.05 ± 0.01 (8)	0.09 ± 0.05 (6)	0.06 ± 0.02 (7)	0.05 ± 0.03 (6)
Liver	Pancreatectomized	1.9 ± 0.6 (8)	4.4 ± 2.5 (9)	1.3 ± 0.7 (7)	0.3 ± 0.1 (5)
	Control	1.6 ± 0.9 (8)	3.5 ± 2.4 (6)	1.9 ± 0.7 (7)	0.3 ± 0.1 (6)
Body retention	Pancreatectomized	4.5 ± 1.6 (9)	6.6 ± 3.3 (9)	3.9 ± 1.1* (7)	1.8 ± 0.6 (6)
	Control	3.7 ± 0.9 (8)	6.9 ± 4.8 (9)	5.3 ± 2.0 (7)	2.3 ± 1.0 (7)

Each value is expressed as per cent of administered dose per gram tissue (average ± S.D.).
( ): No of mice * $P<0.05$

mice was about 20 % lower than that in the control mice at the late intervals (the 48 and 240 hour intervals). With respect to the ^{65}Zn activity in the brain, no difference was found between two groups at the early intervals, while the pancreatectomized group contained about 30 % lower content of ^{65}Zn at the late intervals. The distribution of ^{65}Zn in five parts of the brain is shown by the relative activity in Table 3 and Fig. 1. As described in part I, the characteristic distribution of ^{65}Zn in the brain is more definitely expressed by the relative activity. The relative ^{65}Zn activity in the control mice was almost the same as that in the intact mice in part I.

Table 3. Relative ^{65}Zn activity in various part of the brain of subtotally pancreatectomized mice after subcutaneous injection of $^{65}ZnCl_2$.

Hours after injection		24 (5)	48 (6)	240 (8)	720 (10)
Cerebrum	Pancreatectomized	95 ± 1	97 ± 2	104 ± 3	112 ± 6
	Control	92 ± 1	101 ± 8	100 ± 4	109 ± 7
Ammon's formation	Pancreatectomized	96 ± 6	101 ± 9	120 ± 10*	137 ± 28*
	Control	101 ± 6	103 ± 11	133 ± 8	164 ± 18
Diencephalon	Pancreatectomized	111 ± 11	98 ± 7	91 ± 8	78 ± 7
	Control	102 ± 7	99 ± 6	94 ± 16	77 ± 8
Mesencephlon, Pons & Medulla Oblongata	Pancreatectomized	95 ± 7	106 ± 7	82 ± 6	58 ± 5*
	Control	102 ± 9	102 ± 5	86 ± 5	65 ± 7
Cerebellum	Pancreatectomized	113 ± 13	110 ± 5	109 ± 11	100 ± 6
	Control	111 ± 13	108 ± 8	110 ± 12	104 ± 12

Relative ^{65}Zn activity was calculated as (^{65}Zn activity in each part / mg w.w.) / (^{65}Zn activity in whole brain/ mg w.w.) × 100 %. Each value is expressed as average ± S.D. ( ): No. of mice. *$P<0.05$

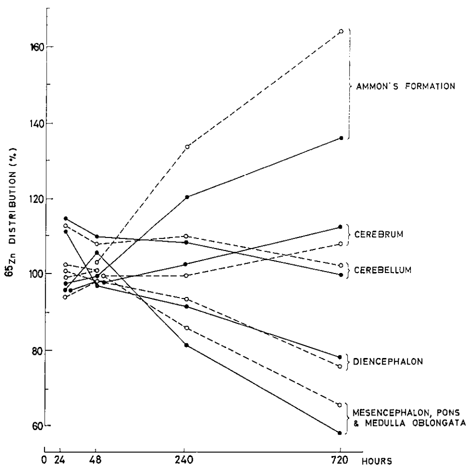

Fig. 1. Relative ^{65}Zn activity in various part of the brain of subtotally pancreatectomized mice after subcutaneous injection of $^{65}ZnCl_2$.
•——• : Pancreatectomized
○·······○ : Control

The most characteristic change following subtotal pancreatectomy occurred in the Ammon's formation. The ^{65}Zn activity in the Ammon's formation in the pancreatectomized mice showed a marked decrease at the late intervals; the average values for the pancreatectomized and control mice were 120% and 133% at the 240 hour interval, and were 137 % and 164 % at the 720 hour interval. These differences were statistically significant at 5 % level. However, these changes are not due to difference in the entry of ^{65}Zn into this part, since any significant changes were not observed at the early intervals (24 and 48 hours). Therefore, this fact indicates that affinity to zinc or zinc metabolism in this part is affected by subtotal pancreatectomy. On the other hand, there was no significant difference in ^{65}Zn activity in the other parts between two groups, except that the ^{65}Zn activity in the mesencephalon, pons & medulla oblongata was slightly lower at the 720 hour interval.

Water content in five parts of the brain at the 720 hour interval after pancreatectomy is shown in Table 4. There was no difference between two groups. Therefore, the observed difference in ^{65}Zn activity produced by subtotal pancreatectomy is not due to difference in water content.

Table 4. Water content in various parts of the brain.

	Cerebrum	Ammon's formation	Diencephalon	Mesencephalon, Pons & Medulla Oblongata	Cerebellum
Pancreatectomized (5)	78.6 ± 0.6	78.9 ± 2.0	78.7 ± 1.1	76.3 ± 0.8	77.7 ± 0.9
Control (5)	79.1 ± 0.7	79.8 ± 2.0	79.7 ± 2.0	76.2 ± 0.8	77.4 ± 0.9

Each value is expressed as per cent of wet weight (average ± S.D.).
( ):No. of mice

Discussion

In the present study, subtotal pancreatectomy caused the following remarkable changes in the distribution of ^{65}Zn; (1) the body retention following gastric gavage decreased at the late intervals; (2) the removal of ^{65}Zn from the blood was delayed; (3) the ^{65}Zn activity in the brain decreased at the 48 hour interval; and (4) the ^{65}Zn distribution in the Ammon's formation showed a marked decrease.

Because of difficulities in the analysis of the small concentration of zinc in tissues, the distribution of ^{65}Zn has been studied as a guide to that of this metal.[37] In this case, it is necessary to control zinc uptake in experimental animals, since the distribution of ^{65}Zn is affected by the administered dose of zinc. In the present study, the administered dose of zinc was practically the same between the pancreatectomized and control mice, since the animals were maintained with pair-feeding technique. Therefore, the observed changes in ^{65}Zn distribution produced by subtotal pancreatectomy indicate that the disturbance of zinc metabolism occurs in the early postoperative period (1 week after operation).

Cotzias et al. (1964)[3] have shown that homeostatic control is operative over the entire zinc pathway, when the absorptive and excretory mechanisms are functioning together. Since the pancreas is an excretory organ of zinc, the excretory mechanism is considered to be disturbed by subtotal pancreatectomy, as suggested by the delayed removal of subcutaneously injected ^{65}Zn from the blood at the 6 hour interval. Therefore, it is considered that the homeostatic control governing zinc metabolism does not operate in the pancreatectomized mice. Thus, it is reasonable to presume that the disturbance of zinc metabolism observed in the early postoperative period continues to occur subsequently.

In the pancreatectomized mice, the ^{65}Zn activity in body retention following gastric gavage and in the blood following subcutaneous injection was lower at the late intervals. Presumably this finding accounts for the decreasement of ^{65}Zn activity in the brain.

It is noteworthy that subtotal pancreatectomy caused a significant decrease of ^{65}Zn content in the Ammon's formation at the late intervals. This indicates that the characteristic prolonged retention of ^{65}Zn in this part becomes less evident following subtotal pancreatectomy. Such change in the Ammon's formation of the pancreatectomized mice is not due to decrease in the entry of ^{65}Zn into this part, since there was no significant change in its uptake at the early intervals. Moreover, it is unlikely that the change in the Ammon's formation is caused by the lower ^{65}Zn activity in whole brain at the 48 hour interval. This is supported by the present observation that the relative activity of ^{65}Zn in the brain of the control mice was almost the same as that of the intact mice in part I, in spite of the different administered dose of ^{65}Zn.

Two possible interpretations are thought to be listed as causes of the decrease in ^{65}Zn distribution in the Ammon's formation. One of these is that the change is produced by the disturbance of zinc metabolism.

Concerning the relationship of zinc metabolism to function of the brain, Hiraoka (1966)[14] reported that zinc deficiency decreased brain excitability and produced a significant decrease in the activity of brain carbonic anhydrase. This fact indicates that the disturbance of zinc metabolism may have some effect on brain metabolism. As described in part I, zinc is thought to have an intimate relationship with the Ammon's formation. Therefore, it is very likely that the disturbance of zinc metabolism caused by subtotal pancreatectomy affects function of the Ammon's formation particularly.

Another possibility is the consideration that pancreatectomy itself affects function of the Ammon's formation without regard to zinc metabolism. Midorikawa et al. (1963)[21] observed that intravenous injection of metal-chelating agents and formalin caused the rapid disappearance of Timm's reaction for zinc in the Ammon's formation. Mc Lardy (1962)[20] reported the same finding following experimental seizures. These observations suggest that such procedures cause functional disturbance of this part so that zinc becomes histochemically undetectable. Thus, the Ammon's formation seems to be easily affected by various types of stress. From the above consideration, one cannot deny the possibility that pancreatectomy itself affects function of the Ammon's formation to the decrease in the prolonged retention of ^{65}Zn. Concerning this problem, further study is necessary.

Summary

The effect of subtotal pancreatectomy on zinc metabolism, especially on the distribution of ^{65}Zn in the brain, was studied in mice. Subtotal pancreatectomy produced the following changes;

1) The body retention of ^{65}Zn was lower than that in the control mice at the late intervals (the 48 and 240 hour intervals) following gastric gavage of ^{65}Zn.

2) The ^{65}Zn activity in the brain showed a significant decrease at the 48 hour interval following gastric gavage or subcutaneous injection.

3) The relative ^{65}Zn activity in the Ammon's formation showed a marked decrease at the late intervals (the 240 and 720 hour intervals) following subcutaneous injection. On the other hand, the activity in other parts did not show significant changes, except that the activity in the mesencephalon, pons & medulla oblongata was slightly lower at the 720 hour interval. These findings indicate that subtotal pancreatectomy particularly affects the Ammon's formation. Its possible mechanisms are discussed from the stand point of zinc metabolism.

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