Neurochemical Considerations on the Alleviating Effect of Caudal Resection of the Pancreas on Epileptic Seizures ; Relationship of Zinc Metabolism to Brain Excitability

Shunji TOKUOKA, Takeshi FUCHIMOTO, Hiroshi HIRAOKA, Masanari TAKASHIMA, Masataka FUJII and Masanori WATANABE
2nd Division. Department of Surgery
(Chief : Prof. S. TOKUOKA)
Yamaguchi University School of Medicine
(Received February 10, 1967)

INTRODUCTION

Although a considerable amount of research has been conducted in an attempt to explain the neurochemical mechanism of seizures, the knowledge of the patho-physiology of epilepsy seems to be limited.

In 1951, Tokuoka,[43] one of the authors, reported that the caudal resection of the pancreas was effective in alleviating the epileptic seizures in 66.7 % of 27 cases of genuine epilepsy, the result being better in childhood. On the basis of this observation, we assumed that the disturbance of zinc metabolism caused by this procedure might have some connection with the alleviating effect.

With respect to zinc in the pancreas, it has been shown that zinc is indispensable to the structural and functional integrity of insulin,[44] while zinc content in the pancreas is many times the amount necessary to account for its insulin content in the form of a zinc salt.[8] Furthermore, Montogomery et al. (1943)[32] have shown that the injected ^{65}Zn is eliminated by way of the external secretion of the pancreas. Therefore it is predictable that the caudal resection of the pancreas produces a disturbance of zinc metabolism.

Zinc is widely distributed in human and other vertebrate organs, and presumably plays a cardinal role in significant aspects of metabolism.[44] However, the biological occurrence and function of zinc are not enough explained as a consequence of poor methodology in investigation. Particulary the biological significance of zinc in the central nervous system is poorly understood. Therefore, there are many difficult problems to be solved in order to verify the above assumption.

Presented in part at the 65th Annual Meeting of Japan Surgical Society, Tokyo, 1965.

This study is undertaken in an attempt to investigate the effect of subtotal pancreatectomy on brain excitability from the standpoint of zinc metabolism. For this purpose, the following problems are experimentally studied : (1) What kinds of effects are produced on brain excitability by subtotal pancreatectomy? (2) Does subtotal pancreatectomy affect zinc metabolism? (3) Is there any sensible relationship between zinc and brain excitability? (4) What neurochemical changes are produced by subtotal pancreatectomy? Is there any similarity between these changes and those produced by zinc deficiency or intoxication? (5) What changes in the endocrine organs are produced by subtotal pancreatectomy? And, as based on the results obtained from these experiments, relation of zinc metabolism to brain excitability in subtotal pancreatectomy is discussed. For the details of study on each problem, readers are referred to recent reports in this laboratory. [10) 19) 41)]

MATERIAL AND METHODS

Except experiments for zinc deficiency, male dd mice (18 to 25g in weight) were used. The animals were maintained on Oriental Chow with free access to food and water.

Treatments of animals

Subtotal pancreatectomy The experimental animals were anesthetized with ether. After laparotomy, the pancreas was exposed and was ligated with a fine silk suture just distal to the superior mesenteric artery. Then, the distal two thirds of the pancreas was removed. The control mice were treated in a fashion similar to that described above, except that the pancreas was only manipulated.

Zinc deficiency According to the method of Nishimura (1953),[37)] new-born mice were nursed by foster in the later stage of lactation. Since zinc content in her milk is markedly lower than that in colostrum, foster sucking mice have come to show a characteristic appearance for zinc deficiency ; alopecia, exfoliation and rosary-like tail were observed in 10 to 15 days post partum. Experiments were performed at 15 days of age.

Systemic zinc intoxication Zinc chloride was given subcutaneously in a daily dose of 50μg for 10 days (subacute intoxication) or in a single dose of 300μg (acute intoxication). The latter experiment was performed 6 hours after the injection. In another series, the animals received zine chloride, dissolved in drinking water at a concentration of 0.02%, for 4 weeks (chronic intoxication).

Determination of brain excitability

The electroshock seizure threshold (EST) and the maximal electroshock seizure duration (MES) were measured using Woodbury and Davenport's apparatus (1952).[48)] The electrical stimulus was delivered for 0.2 sec. through silver

electrode placed over the eyes. The EST_{50} was calculated by the method of Litchfield and Wilcoxon (1949).[24] For determining MES, a supramaximal stimulus of 50 mA or 100 mA (for the zinc deficient mice) was applied, and the duration of the tonic extensor phase was measured as the most representative index of MES.

Chemical studies

The animals were killed by decapitation. For determination of brain electrolytes, the fresh whole brain was homogenized with 10% trichloracetic acid and the supernatant was used for assay. Serum was obtained by centrifugation of blood from the carotid artery. Potassium and sodium were determined by flame photometry. Calcium was analyzed by the method of Appleton et al. (1959).[1] Carbonic anhydrase activity was determined by Nishimura's modification (1963)[38] of Maren's pH changing method (1960).[25] For determining water content of brain tissue, the sample was dried in an oven at 110°C for 12 hours.

Isotopical studies

A single dose of about 4μc of $^{65}ZnCl_2$, obtained from New England Nuclear Corp., was administered by subcutaneous injection or gastric gavage. At various intervals thereafter, tissue and organ samples were analyzed for ^{65}Zn content in a well-type gamma scintillation counter. Prior to measurement of the body retention of ^{65}Zn, each organ and carcass were dissolved in concentrated H_2SO_4 after separation of the content of the gastrointestinal tract. No decay corrections were made. For determining the chemical form of ^{65}Zn in the brain, whole brain was homogenized with ice-cold phosphate buffer solution at pH 7.2 and was dialyzed against the same solution. Lipids were extracted by the method of Folch et al. (1951).[9]

Intracerebral injection technique

Intracerebral injection was performed by our modification[10] of the method of Haley and McCormick (1957).[14] Reagents were dissolved in Ringer's solution. The injection volume was 0.01 or 0.02 ml.

Histological studies

Tissue specimens were fixed in 10% formalin and histological sections were stained with hematoxylin-eosin. Timm's silver-sulphide method[42] was used for the histochemical display of zinc.

EXPERIMENTAL RESULTS

1. The effect of subtotal pancreatectomy on brain excitability

Changes in brain excitability following subtotal pancreatectomy are shown in Fig. 1. After a period of 2 to 3 weeks, the EST of the pancreatectomized mice was scarcely changed, while the MES was significantly shortened. In the

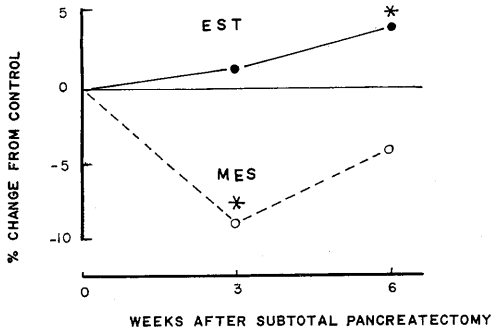

Fig. 1. Effect of subtotal pancreatectomy on brain excitability. Changes in brain excitability are expressed as the percentage of control values. EST, electroshock seizure threshold : MES, maximal electroshock seizure duration.
* Statistically significant.

course of 6 weeks, the EST was 5% higher than that of controls, and the difference was statistically significant at 1% level. On the other hand, the MES was shortened to minus 4%. It has been shown that EST is influenced by body weight.[48] However it is not applicable in this study, since the body weight of subtotally pancreatectomized mice showed no significant difference compared with that of controls.[41] From the above findings it is evident that subtotal pancreatectomy affects brain excitability ; both the increased EST and the shortened MES indicate the decreased brain excitability.

2. The effect of subtotal pancreatectomy on zinc metabolism

Because of difficulties in the analysis of the small concentrations of zinc in tissues, the distribution of ^{65}Zn has been studied as a guide to that of this metal.[44] ^{65}Zn was administered 1 week after subtotal pancreatectomy.

The distribution of subcutaneously injected ^{65}Zn in the blood and brain at various intervals up to 240 hours is shown in Fig. 2. The removal of ^{65}Zn from the blood was delayed in the pancreatectomized mice ; the ^{65}Zn content at the 6 hour interval was about twice higher than that of controls. However, it fell below the control value after 18 hours. On the other hand, there was a significant decrease in the ^{65}Zn content of the brain at the 48 hour interval ; the average value for the pancreatectomized and control mice was 1.6 % and 2.3 % respectively.

The time course of tissue distribution of orally administered ^{65}Zn is shown in Fig. 3. At the 48 and 240 hour intervals, the body retention of ^{65}Zn in the pancreatectomized mice was about 20 % lower than that in control mice. With respect to the ^{65}Zn content in the brain, no difference was observed between the two groups at the early intervals (12 and 24 hours), while the

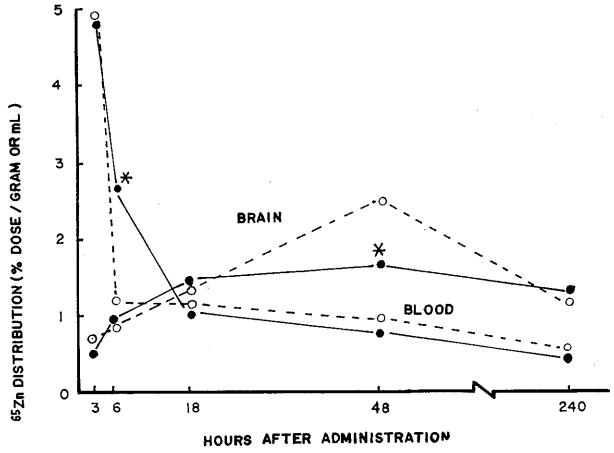

Fig. 2. Distribution of subcutaneously injected $^{65}ZnCl_2$ in the brain and blood of subtotally pancreatectomized mice. (•), pancreatectomized ; (◦), control.
* Statistically significant.

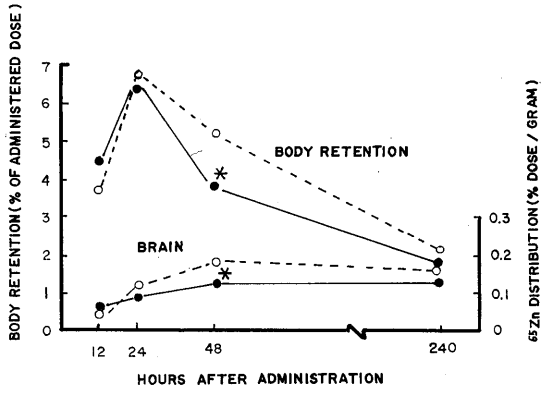

Fig. 3. Distribution of orally administered $^{65}ZnCl_2$ in subtotally pancreatectomized mice. (•), pancreatectomized ; (◦), control.
* Statistically significant.

pancreatectomized group contained about 30% lower content of ^{65}Zn at the late intervals (48 and 240 hours).

For lack of determination of zinc content, the above data could not be culculated as a guide to isotope turnover rates. Cotzias and Papavasiliou (1964)[4] have mentioned that distortions of the partition of ^{65}Zn, the ratio of organ to whole body content, reflect the turnover rates of individual organs. As recalculated from Fig. 3, the partition of orally administered ^{65}Zn in the brain was increased as time lapsed. Especially the increase was slower in the

pancreatectomized mice.

From the above findings, it is evident that subtotal pancreatectomy causes the disturbance of zinc metabolism.

Since brain function varies widely with the parts, the distribution of ^{65}Zn was studied in five parts, i. e., the hippocampus and dentate gyrus, the cerebrum excluding the above part, diencephalon, the cerebellum and the remaining part (midbrain, pons and medulla oblongata). As shown in Fig. 4, the ^{65}Zn content in each part had practically the same value at the 48 hour interval. At the late intervals (240 and 720 hours), however, the different parts of the brain were divided according to their ^{65}Zn content into three groups ; the first group comprised the diencephalon and the remaining part showed a lower content, while the second group consisted of the cerebrum and the cerebellum showed a higher one. The highest ^{65}Zn content in the hippocampus and dentate gyrus was most characteristic ; the content in this part was about three times as high as in the remaining part at the 720 hour interval. It has been shown that zinc is histochemically detectable in the hippocampus and dentate gyrus.[26) 42)] Therefore, the highest uptake of ^{65}Zn seems to be closely related to the pathophysiology of zinc in this region. It is noteworthy that subtotal pancreatectomy caused a significant decrease of the ^{65}Zn content in the hippocampus and dentate gyrus at the late intervals. Such a characteristic change is not due to

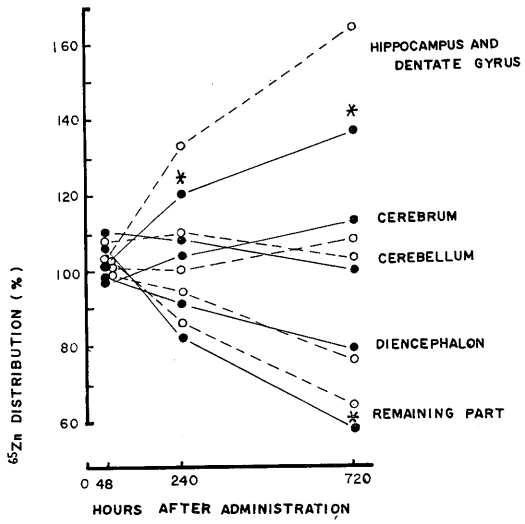

Fig. 4. Distribution of subcutaneously injected $^{65}ZnCl_2$ in different parts of the brain following subtotal pancreatectomy. The results are given as the following ratio : (^{65}Zn content of each part/mg w.w.)/ (^{65}Zn content of whole brain/mg w.w.). (•), pancreatectomized ; (○), control.
* Statistically significant.

the decreased entry of ^{65}Zn into this part, since no significant change in its uptake was observed at the early interval (48 hours). Rather, this finding indicates that zinc metabolism in this part is affected by subtotal pancreatectomy.

On the other hand, ^{65}Zn in the brain was scarcely dialyzable at pH 7.2 and was not contained in total lipid extracts. Therefore, ^{65}Zn in the brain is considered to be bound to proteins in the form of metalloprotein or metal-protein complex.

3. Relationship of zinc to brain excitability

Relationship of zinc to brain excitability has been investigated in this laboratory.[16)19)34)35)]

a) The effect of zinc deficiency or intoxication on brain excitability

Nagasue (1957)[34)35)] observed that the cardiazol-seizure threshold of the sucking mice was increased by zinc deficiency, while zinc intoxication decreased it.

Changes in EST and MES produced by zinc deficiency or intoxication are shown in Fig. 5 and 6. Zinc deficiency increased EST, while zinc intoxication decreased it, being consistent with the observation of Nagasue. The changes in EST varied from 5 to 13% in either direction. Such changes are slight but quite significant, since even the EST of ep mouse, a convulsive strain of mouse, is less than 20% lower than that of dd mouse.[40)] The MES of the zinc deficient mice was shortened by 18%. This finding appears to be compatible

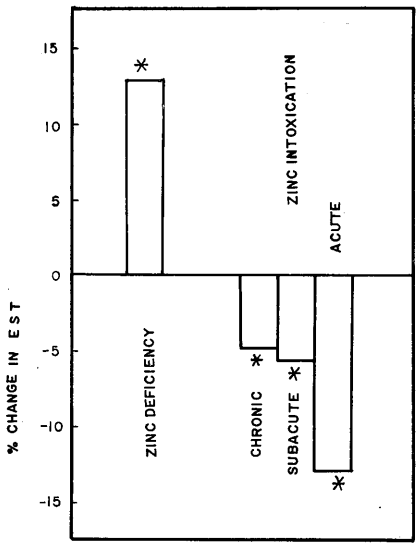

Fig. 5. Effect of zinc deficiency or intoxication on EST. Changes in EST are expressed as the percentage of control values. Experimental conditions are described in the text.
* Statistically significant.

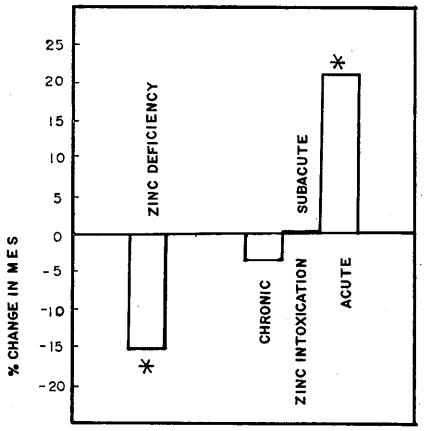

Fig. 6. Effect of zinc deficiency or intoxication on MES. Changes in MES are expressed as the percentage of control values. Experimental conditions are described in the text.
* Statistically significant.

with the observation that zinc deficient calves would not respond to stimuli intended to cause vigorous exercise.[30] The effect of zinc intoxication on MES was variable ; the MES was markedly lengthened in acute intoxication, while subacute and chronic intoxication did not produce a significant change.

It is worthy of mention that the effect of zinc deficiency on brain excitability was determined in the developmental stage of the brain, when the EST is markedly decreased up to about 15 days of age and is slowly increased thereafter,[46] while the tonic extensor component of MES is not elicited until the later stage.[31] To be exact, therefore, the effect of zinc deficiency presented here should be interpreted as the effect on the development of seizure activity.

b) *Changes in EST and initiation of seizures following intracerebral injection of zinc*

Intracerebral injection of 0.01 ml of Ringer's solution containing zinc chloride (pH 5.4) produced an intensive effect on EST. As shown in Fig. 7, the EST was decreased as the concentration of zinc was increased ; the decrease amounted to 15% at a concentration of 3 mM. This EST-lowering effect was more pronaunced than that of cobalt and iron, 6% and 0% at 3mM.

With still higher concentration (4 mM or more), zinc produced a tonic seizure, essentially similar to the maximal seizures produced by electroshock,[48] with a latency of about 50 sec. Since copper (II) produced a different type of seizure response and several heavy metals had little effect on behavior at 5 mM, the seizure produced by zinc is considered to be rather specific for this metal.

c) *Changes in EST following intracerebral injection of metal-chelating agents.*

Histochemists have shown that zinc is displayed particulary in the hippocampus

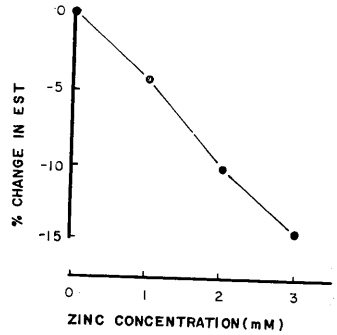

Fig. 7. Effect of intracerebrally administered zinc on EST. Changes in EST are expressed as the percentage of control value. EST test was applied 50 sec. after intracerebral injection of zinc. Injection volume was 0.01 ml. Control value = 6.1 mA (95% confidence limits : 6.5−5.7 mA)

and dentate gyrus.[26)27)28)42)] It is very likely that this zinc is most easily affected by metal-chelating agents, since histochemically detectable metals presumably exist in the form of metal-protein complex. Midorikawa et al. (1963)[29)] observed that intravenous injection of metal-chelating agents produced the disappearance of Timm's reaction for zinc in this region. Thus, a low concentration of calcium disodium ethylene-diamine-tetraacetate (Ca-EDTA) and sodium diethyldithiocarbamate (SDDC) was intracerebrally injected and changes in EST were studied, in order to examine the relationship of zinc in the hippocampus and dentate gyrus to brain excitability.

The result obtained is shown in Fig. 8. On the injection of Ca-EDTA the EST

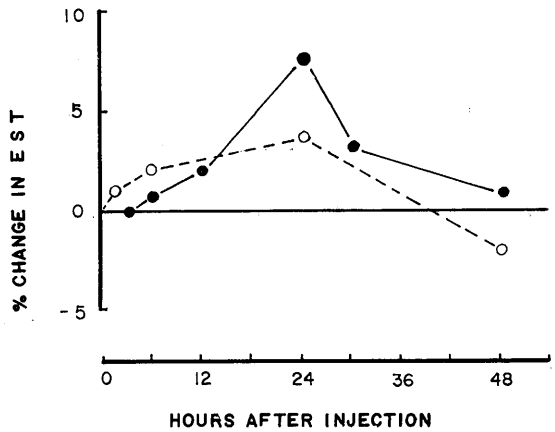

Fig. 8. Effect of intracerebrally administered metal-chelating agents on EST. Changes in EST are expressed as the percentage of control value at each time. Injection volume was 0.02 ml. (•), 0.2% calcium disodium ethylene-diamine-tetraacetate ; (○), 0.1% sodium diethyldithiocarbamate.

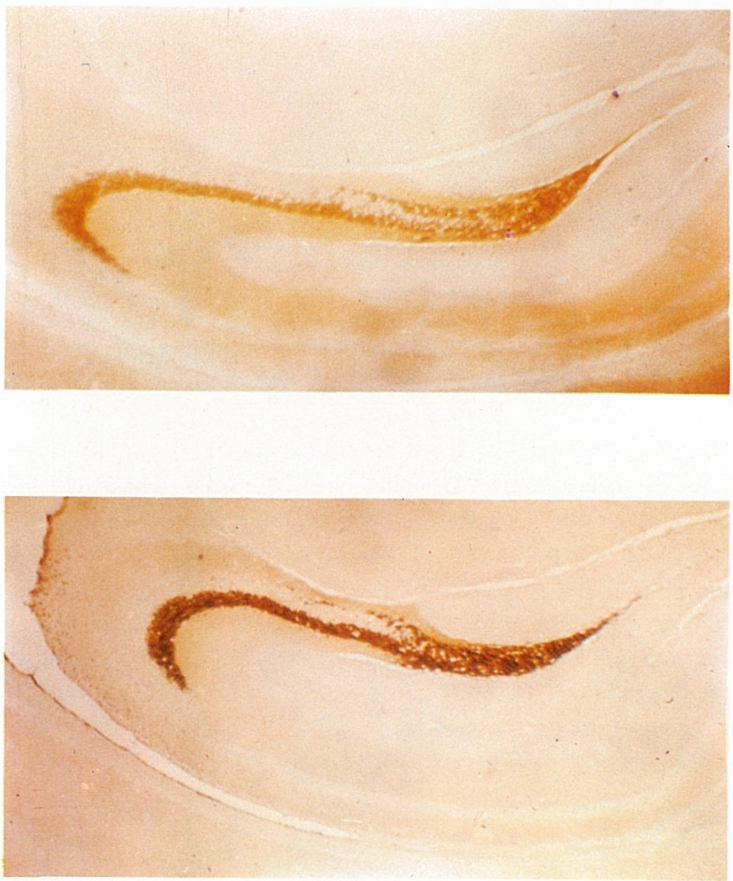

Fig. 9. Positive Timm's reaction in the hippocampus and dentate gyrus. Intensity of the reaction 24 hours after intracerebral injection of Ca-EDTA (lower) is diminished as compared with that in the control (upper).

rose gradually and reached a peak of 8% after 24 hours, while SDDC also produced a slight increase in EST. On the other hand, histochemical study revealed that the zinc reaction in the hippocampus and dentate gyrus was diminished below the control 24 hours after the injection of Ca-EDTA, as shown in Fig. 9. SDDC did not produce any change in this reaction, probably due to the formation of a water-insoluble chelate compound.

4. Neurochemical changes produced by subtotal pancreatectomy, zinc deficiency or intoxication.

Although neurochemical mechanisms governing brain excitability are diverse, the present study deals with only the problems in relation to zinc. It has been shown that brain carbonic anhydrase, a zinc metalloenzyme, is closely related to the brain excitability.[2)12)23)31)38)] On the other hand, Fuchimoto[10)] suggested that zinc may have some effects on the electrolytes distribution in the brain, whose relation to the brain excitability has been studied extensively by Woodbury and his colleagues.[46)47)]

The effects of subtotal pancreatectomy, zinc deficiency or intoxication on brain carbonic anhydrase, electrolytes and serum electrolytes are summarized in Table 1. The direction of the arrow indicates the direction on the change in response.

Table 1. Effect of subtotal pancreatectomy, zinc deficiency or intoxication on electrolyte metabolism and brain carbonic anhydrase (CAH)

Group	Brain					Serum	
	H_2O	Na	K	Ca	CAH	Na	K
Subtotal Pancreatectomy 3W	↔	↔	↔	↑	↓	↔	↔
Subtotal Pancreatectomy 6W	↔	↔	↔	⇑	⇓	⇑	↓
Zinc Deficiency	↔	↔	↔	↑	↓	↔	↔
Zinc Intoxication Acute	↓	↔	↔	↔	↔	↓	↑
Zinc Intoxication Subacute	↓	↑	↔	↓	↔	↔	↑

The direction of the arrow indicates the direction of change in response. Experimental conditions are described in the text. ↔, not changed ; ↓↑, statistically not significant ; ⇓ ⇑, statistically significant.

Brain carbonic anhydrase was significantly decreased 3 and 6 weeks after subtotal pancreatectomy. Zinc deficiency produced a significant decrease in the enzymatic activity, while zinc intoxication had practically no effect. The decrease in brain carbonic anhydrase produced by subtotal pancreatectomy and zinc deficiency appears to be related to the shortened MES, since acetazoleamide, a

potent carbonic anhydrase inhibitor, shortens MES without affecting EST.[12)31)]

Serum Na concentration following subtotal pancreatectomy was significantly increased in 6 weeks, although it was unchanged in 3 weeks. Acute zinc intoxication produced a significant decrease in serum Na, while zinc deficiency had little effect. On the other hand, there were little or no changes in serum K level in each case. Brain Na and K concentration were unchanged except for an increase in brain Na in subacute intoxication.

Woodbury (1954)[47)] has shown that the concentrations of plasma Na and/or of intracellular brain Na and/or the ratio of extracellular to intracellular brain Na is correlated with the level of brain excitability ; increased plasma Na concentration, decreased intracellular brain Na concentration or an increased ratio between the two results in depressed brain excitability (increased EST), while the opposite changes result in enhanced brain excitability (decresed EST). Therefore, changes in electrolytes presented here are consistent with those in brain excitability.

Brain Ca concentration following subtotal pancreatectomy was significantly increased in 6 weeks. This effect was similar to that produced by zinc deficiency. Acute zinc intoxication had practically no effect on brain Ca, while subacute intoxication decreased it significantly. Since Ca ion is considered to regulate the membrane permeability to Na and K ions in the excitable tissues,[3)39)] such changes in brain Ca may have some connection with those in brain excitability.

The above findings may be summarized as follows : (1) subtotal pancreatectomy produced an increase in brain Ca and serum Na, and a decrease in brain carbonic anhydrase ; (2) zinc affected electrolytes in the brain and serum, while brain carbonic anhydrase was decreased in zinc deficiency alone ; (3) in general, the effect of subtotal pancreatectomy on brain carbonic anhydrase and electrolytes was similar to that of zinc deficiency ; and (4) these changes were compatible with those in brain excitability.

5. The effect of subtotal pancreatectomy on the endocrine organs

It has been shown that subtotal pancreatectomy does not result in the development of diabetes mellitus.[15)41)] In the present study blood sugar level was within the normal range, indicating that the effect of subtotal pancreatectomy on brain excitability is not due to hyperglycemia.

On the other hand, the histological study showed that the thyroid gland of the subtotal pancreatectomized mice was microfollicular and atrophic, while the adrenal gland and hypophysis appeared to be normal. These findings are consistent with the observation of Nihail and Ionescu (1965)[36)] in pigeons.

DISCUSSION

The present study showed that subtotal pancreatectomy decreased brain excitability ; the EST was increased and the MES was shortened. In order to establish that this effect is produced by the disturbance of zinc metabolism, at least the following two points should be proved : (1) subtotal pancreatectomy causes the disturbance of zinc metabolism ; and (2) zinc metabolism has relation to brain excitability.

As shown in the present study, subtotal pancreatectomy produced remarkable changes in the distribution of ^{65}Zn : (1) a decrease in the body retention following oral administration ; (2) a decrease in the brain with the peak change at the 48 hour interval ; and (3) a marked disturbance in the partition of the brain. These findings suggest that subtotal pancreatectomy causes the derangement of zinc metabolism in the early postoperative period (1 week after the operation). Cotzias and Papavasiliou (1964)[4] have demonstrated that homeostatic controls are operative over the entire zinc pathway only when the absorptive and the excretory mechanisms are functioning together. Since the pancreas is an excretory organ of zinc,[32] the excretory mechanism is considered to be affected by subtotal pancreatectomy. The delayed removal of subcutaneously injected ^{65}Zn from the blood appears to confirm the above consideration. Therefore it may be predicted safely that the zinc metabolism is continuously perturbed as a result of the disturbance of homeostatic controls.

The increased EST with shortened MES in zinc deficiency and the decreased EST in zinc intoxication clearly demonstrate that zinc exerts some effects on the mechanisms governing brain excitability. Furthermore, as shown in the present study, intracerebrally administered zinc can produce a threshold-lowering effect and act as a convulsant by its direct action on the brain, although the effect of exogenous zinc may be different from the biological action of zinc.

Such an intensive effect of zinc on brain excitability leads us to the concept that the disturbance of zinc metabolism may be participated with the decreased brain excitability in subtotal pancreatectomy.

The role of zinc in brain excitability is scarcely explained. Since zinc is presumably combined with proteins, as shown in the present isotopical study, it is reasonable to believe that zinc acts on the brain as the metalloenzyme or metal-protein complex. In the former zinc and protein is firmly bound so that the two can be thought of as an entity, while in the latter zinc combines reversibly with protein.[45] With respect to zinc metalloenzyme, it is generally accepted that brain carbonic anhydrase is closely related to the brain excitability.[2][12][23][31] As based on the current concept that changes in the activity of brain carbonic anhydrase affect MES,[12][31] the shortening of MES following

subtotal pancreatectomy appears to be connected with the decrease in the enzymatic activity. Some investigators[5)6)20)] showed that no significant lowering of the enzymatic activity in tissues was encountered in adult zinc deficient animals, in contrast to a significant decrease in the brain of the zinc deficient sucking mice. On the other hand, the present study showed that zinc intoxication had little effect on brain carbonic anhydrase. Therefore it seems unlikely that the decrease in the enzymatic activity of the pancreatectomized mice brain is due to the direct effect of the disturbance of zinc metabolism.

Another role of zinc in brain excitability was suggested by Fuchimoto[10)]; zinc may affect the neurochemical mechanism of seizures through a change in the intracellular or extracellular distribution of brain electrolytes. This effect is presumably due to an action on functions associated with the cell membrane. As based on the present observation that zinc deficiency or intoxication had some effects on electrolytes in the brain and serum, it may be possible that an increase in serum Na and brain Ca following subtotal pancreatectomy is produced by the disturbance of zinc metabolism.

The changes in brain excitability and its neurochemistry following subtotal pancreatectomy were nearly consistent with those in zinc deficiency. This finding, together with a decrease in the body retention of orally administered ^{65}Zn in the early postoperative period, suggests that subtotal pancreatectomy causes the development of zinc deficiency. Furthermore, a characteristic decrease in the retention of ^{65}Zn in the hippocampus and dentate gyrus suggests that zinc deficiency occurs prominently in the specific part, even though not manifest in the whole brain. Probably such characteristic change does not occur in dietary zinc deficiency.

It has been shown that the hippocampus and dentate gyrus may be an important agency of epileptic seizures.[11)13)] Particulary it has been suggested that the seizure activity observed in animals originates in the limbic system.[21)23)]

Although zinc histochemically detected in this region has been shown to disappear following experimental seizures, its relationship to brain excitability is not established. Euler (1961)[7)] has electrophysiologically shown that the synapses of the mossy fibers in the hippocampus and dentate gyrus are blocked by local application of H_2S, reacting on zinc. His observation suggests that the zinc-containing substances may play an important role in the transmission of excitation.

With respect to the neurochemical significance of zinc in the hippocampus and dentate gyrus, Klee and Liefländer (1965)[22)] denied its close relationship to carbonic anhydrase. Recently, Hayashi et al.[17)18)] have shown that there are two cortical pathways of producing epileptic seizure : pallial seizure system and limbic seizure system, and the former is inhibited by γ-amino-β-hydroxy-

butyric acid, but the latter, by homocarnosine and carnosine. On the basis of the observation that zinc potently activates carnosinase,[16)44)] it seems likely that the effect of zinc on such enzymes may produce changes in brain excitability.

The present study indicated that intracerebral injection of metal-chelating agents produced an increase in EST, which was accompanied by the diminution of Timm's reaction for zinc in the hippocampus and dentate gyrus. Metal-chelating agents do not necessarily act on zinc alone, while SDDC produces some toxic effect on the nerve cells.[29)] Therefore these findings should be evaluated prudently.

Considering the above discussion, however, the present experimental findings seem to provide some support for the view that changes in zinc metabolism in the hippocampus and dentate gyrus can produce changes in brain excitability ; decrease in zinc causes decrease in brain excitability. And, if this view is accepted, it is reasonable to consider that the characteristic zinc deficiency in the hippocampus and dentate gyrus, as suggested by the present isotopical data, may have some relation to the decreased brain excitability following subtotal pancreatectomy.

The participation of the endocrine system in the effect of subtotal pancreatectomy on brain excitability deserves another discussion.

Woodbury (1954)[47)] has shown that the various altered endocrine states affect EST and electrolyte metabolism of the brain. The present study revealed that the thyroid gland was microfollicular and atrophic, suggesting hypofunction. Since hypothyroidism has been shown to decrease brain excitability,[47)] it is possible that the hormonal disturbance is participated in the increased EST following subtotal pancreatectomy. Presumably dysfunction of the other endocrine organs, in spite of lack of histological changes, may be produced by this procedure.

From the present experimental findings and the foregoing discussion, it may be concluded that the decrease in brain excitability following subtotal pancreatectomy is closely connected with the disturbance of zinc metabolism, although the participation of hormonal disturbances cannot be denied. Furthermore this interpretation may be applied to the clinical observation, the alleviation of epileptic seizures following the caudal resection of the pancreas, although there are several differences between the experimental and clinical situations : (1) species ; (2) initial level of brain excitability (normal level vs. active seizure discharge) ; and (3) brain excitability vs. epilepsy.

SUMMARY

A series of experiments were performed in order to verify our assumption that the alleviating effect of the caudal resection of the pancreas on the epileptic

seizures may have some connection with the disturbance of zinc metabolism. For this purpose the following problems were investigated : (1) the effect of subtotal pancreatectomy on zinc metabolism, brain excitability and its neurochemistry ; (2) relationship of zinc metabolism to brain excitability and its neurochemistry ; and (3) histological changes in the endocrine organs following subtotal pancreatectomy.

Subtotal pancreatectomy caused a marked disturbance of zinc metabolism in the early postoperative period, as shown in the following isotopical data : (1) a decrease in the body retention of ^{65}Zn ; (2) a decrease in the distribution of ^{65}Zn in the brain ; and (3) a decrease in the retention of ^{65}Zn in the hippocampus and dentate gyrus. The intensive effect of zinc deficiency or intoxication on brain excitability suggests that such disturbances of zinc metabolism may produce changes in brain excitability. The changes in brain excitability and its neurochemistry following subtotal pancreatectomy were nearly consistent with those in zinc deficiency (i. e., increased EST and shortened MES, decreased brain carbonic anhydrase and increased brain Ca). This finding is compatible with the isotopical data, indicating that subtotal pancreatectomy causes the development of zinc deficiency.

Intracerebral injection of metal-chelating agents caused both an increase in EST and the diminution of Timm's reaction for zinc in the hippocampus and dentate gyrus. From this observation and the discussion in this paper, it seems likely that the characteristic decrease in the retention of ^{65}Zn in the hippocampus and dentate gyrus following subtotal pancreatectomy has some effect on the neurochemical mechanism of seizures in this region.

The sum of the results obtained indicates that the effect of subtotal pancreatectomy on brain excitability is closely related to the disturbance of zinc metabolism, although the participation of hormonal disturbances such as hypothyroidism cannot be denied.

REFERENCES

1) Appleton, H. D., West, M., Mandel, M. and Sala, A. M. : The rapid determination of calcium in biological material. *Clin. Chem.,* **5** : 36-44, 1959.
2) Ashby, W., Garzoli, R. F. and Schuster, E. M. : Relative distribution patterns of three brain enzymes, carbonic anhydrase, choline esterase and acetyl phosphatase. *Am. J. Physiol.,* **170** : 116-120, 1952.
3) Brink, F. : The role of calcium ions in neural processes. *Pharmacol. Rev.,* **6** : 243-298, 1954.
4) Cotzias, G. C. and Papavasiliou, P. S. : Specificity of zinc pathway through the body : homeostatic considerations. *Am. J. Physiol.* **206** : 787-792, 1964.
5) Day, H. G. and Mc Collum, E. V. : Effects of acute dietary zinc deficiency in the rats *Proc. Soc. Exptl. Biol. Med.,* **45** : 282-284, 1940.

6) De Conciliis, U. : Carbonic anhydrase of the lens in rabbits kept on a zinc free diet. *Arch. Ottal.,* **62** : 67-72, 1958. Cit. in *Excerpta Medica.*

7) Euler, C. von : On the significance of the high zinc content in the hippocampal formation. *Colloques internat. du Centre Natonal de la Recherche Scientifique. physiol. de l'hippocampe,* **107** : 1-10, 1961

8) Fisher, A. M. and Scott, D. A. : Zinc content of bovine pancreas. *Biochem. J.,* **29** : 1055-1058, 1935.

9) Folch, J., Ascoli, I., Less, M., Meath, J. A. and Le Baron, F. N. : Preparation of lipide extracts from brain tissue. *J. Biol. Chem.,* **191** : 833-841, 1951

10) Fuchimoto, T. : The effect of zinc on the central nervous system. To be published.

11) Gibbs, E. L., Gibbs, F. A. and Fuster, B. : Psychomotor epilepsy. *Arch. Neurol. Psychiat.,* **60** : 331-339, 1948.

12) Gray, W. D., Rauh, C. E., Osterberg, A. C. and Lipchuck, L. M. : The anticonvulsant actions of methazolamide (a carbonic anhydrase inhibitor) and diphenylhydantoin. *J. Pharmacol. Exptl. Therap.,* **124** : 149-160, 1958.

13) Green, J. D. and Shimamoto, T. : Hippocampal seizures and their propagation. *Arch. Neurol. Psychiat.,* **70** : 687-702, 1953.

14) Haley, T. J. and McCormick, W. G. : Pharmacological effects produced by intracerebral injection of drugs in the conscious mause. *Brit. J. Pharmacol.,* **12** : 12-15, 1957.

15) Hallman, G. L. and gordan, G. L. : Subtotal pancreatectomy and growth of young mice. *J. A. M. A.,* **191** : 233-234, 1965.

16) Hanson, H. T. and Smith, E. L. : Carnosinase : an enzyme of swine kidney. *J. Biol. Chem.,* **179** : 789-801, 1949.

17) Hayashi, T., Takagaki, G. and Takada, A. : On the presence of carnosine, homocarnosine, anserine in the central nervous system of various animal species. *Neurologia medico-chirurgica,* **5** : 77, 1963.

18) Hayashi, T. : Boundary between neurophysiology and neurochemistry of convulsion. *J. Physiol. Soc. Japan,* **25** : 512, 1963. (Japanese)

19) Hiraoka, H. : The role of zinc in brain excitability : the effect of disturbed zinc metabolism on brain excitability and relationship of brain carbonic anhydrase (zinc metalloenzyme) to brain excitability. *Bull. Yamaguchi Med. School,* **13** : 269-280, 1966.

20) Hove, E, Elvehjem, C. A. and Hart, E. B. : The relation of zinc to carbonic anhydrase. *J. Biol. Chem.,* **136** : 425-434, 1940.

21) Jinnai, D., Mori, A., Kawanishi, M., Sugiu, R., Masukawa, S. and Miyake, S. : Studies on homocarnosine as a inhibitory substance in cerebral cortex. *Neurologia medico-chirurgica,* **5** : 78, 1963.

22) Klee, M. R. and Liefländer, M. : Über das Vorkommen von Zink und Carbonat-Hydro-lyase im Kaninchenhirn. *Hoppe-Seyler' Z. Physiol. Chem.,* **341** : 143-145, 1965.

23) Koch, A. and Woodbury, D. M. : Effects of carbonic anhydrase inhibition on brain excitability. *J. Pharm. Exptl. Therap.,* **122** : 335-342, 1958.

24) Litchfield, J. T., Jr. and Wilcoxon, F. : A simplified method of evaluating dose-effect experiments. *J. Pharmacol.,* **96** : 99-113, 1949.

25) Maren, T. H. : A simplified micromethod for the determination of carbonic anhydrase and its inhibitors. *J. Pharmacol. Exptl. Therap.,* **130** : 26-29, 1960.

26) Maske, H. : Uber den topochemischen Nachweis von Zink im Ammonshorn verschiedener Säugetiere. *Naturwissenschaften,* **42** : 424, 1955.

27) Mc Lardy, T. : Zinc enzymes and the hippocampal mossy fibre system. *Nature,* **194** : 300-302, 1962.

28) Mc Lardy, T. : Pathological zinc-rich synapses. *Nature.* **195** : 1315-1316, 1962.

29) Midorikawa, O., Okuda, T., Takahashi, K. and Takahashi, C. : Histochemical studies on

the limbic system. (II) : Studies on the histochemical changes in the area of limbic system after administration of the chemical compounds. *Tr. Soc. Path. Jap.,* **52** : 151, 1963. (Japanese)

30) Miller, W. J., Pitts, W. J., Clifton, C. M. and Morton, J. D. : Influence of zinc deficiency on feed deficiency, behavior, graying, tissue zinc and enzymes in the male bovine. *Fed. Proc.,* **24** : 171, 1965.
31) Millichap, J. G. : Development of seizure patterns in newborn animals. Significance of brain carbonic anhydrase. *Proc. Soc. Exptl. Biol. Med.,* **96** : 125-129, 1957.
32) Montgomery, L., Sheline, G. E. and Chaikoff, I. L. : The elimination of administered zinc in pancreatic juice, duodenal juice, and bile of the dog as measured by its radioactive isotope (Zn^{65}). *J. Exptl. Med.,* **78** : 151-159, 1943
33) Mori, A., Sugiu, R., Masukawa, S., Miyake, S. and Kosaka, M. : Research on homocarnosine (γ-aminobutyryl-L-histidine). *Recent advance in research of the nervous system,* **7** : 896-899, 1963. (Japanese)
34) Nagasue, H. : Experimental studies on the biological significance of zinc in the central nervous system. I. Threshold value of convulsion and the histological findings of the brain in the zinc deficient animals. *Jahresbericht d. Kurashiki-Zentral hospitals,* **27** : 155-181, 1957. (Japanese)
35) Nagasue, H. : Ditto. Threshold value of convulsion and histological findings of the brain in the animals intoxicated with zinc. *Ibid.,* **27** : 182-200, 1957. (Japanese)
36) Nihail, N. and Ionescu, M. : Folgeerscheinungen nach pankreasentfernung bei Vögeln. *Naturwissenschaften,* **50** : 650, 1963.
37) Nishimura, H. : Zinc deficiency in suckling mice deprived of colostrum. *J. Nutrit.,* **49** : 79-97, 1953.
38) Nishimura, T., Tanimukai, H. and Nishimura, K. : Distribution of carbonic anhydrase in human brain. *J. Neurochem.,* **10** : 257-261, 1963.
39) Shanes, A. M. : Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmacol. Rev.,* **10** : 59-273, 1958.
40) Takahashi, R., Kariya, T., Kobayashi, K., Toru, M., Kobayashi, T. and Nasu, T. : A study on the recovery process of brain dysfunction induced by repetition of electric stimulation and anoxia. *Recent advance in research of the nervous system,* **6** : 139-143, 1962. (Japanese)
41) Takashima, M. : The mechanism of alleviation of the epileptic seizures following the caudal excision of the pancreas : its effects on the brain excitability, electrolytes and brain carbonic anhydrase. *Bull. Yamaguchi Med. School,* in press.
42) Timm, F. : Zur Histochemie des Ammonshorngebietes. *Z. Zellforsch.,* **48** : 548-555, 1958.
43) Tokuoka, S. : Patho-physiological studies on epilepsy. *Acta Schol. Med. Univ. Kioto,* **29** : 15-30, 1951.
44) Vallee, B. L. : Biochemistry, physiology and pathology of zinc. *Physiol. Rev.,* **39** : 443-490, 1959.
45) Vallee, B. L. : Zinc and metalloenzymes. *Advances in protein chemistry.* **10** : 317-384, 1955.
46) Vernadakis, A. and Woodbury, D.M. : Effects of diphenylhydantoin on electroshock seizure thresholds in developing rats. *J. Pharmacol. Exptl. Med.,* **148** : 144-150, 1965.
47) Woodbury, D. M. : Effect of hormones on brain excitability and electrolytes. *Recent progress in hormone research,* Pincus, G., ed. **10** : 65-107, Academic Press inc., New York, 1954.
48) Woodbury, L. A. and Davenport, V. D. : Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. *Arch. Int. Pharmacodyn.,* **92** : 97-107, 1952.