The Effect of Zinc on the Adrenal Gland with Special Reference to the Increasing Effect on Brain Excitability

Yoshirou SANTOKI
Second Division, Department of Surgery, Yamaguchi University School of Medicine.
(*Director: Ex-Prof. Dr. Shunji Tokuoka*)
(Recevied December 23, 1974)

INTRODUCTION

The effect of zine on brain excitability was suggested by Tokuoka[1] in 1951, based on the observation that the caudal resection of the pancreas was effective in alleviating epileptic seizures in genuine epilepsy. On the other hand, Peters[2] observed that, in patients with some types of porphyria, zincuria occurred simultaneously with seizures, which were markedly improved by administration of chelating agents. However, the effect of zinc on the central nervous system is only vaguely understood.

In 1967, Fuchimoto et al.[3] reported that systemically or intracerebrally injected zinc markedly decreased the electroshock seizure threshold (EST) in mice. They showed that the EST-lowering effect produced by intracerebral injection of zinc was not influenced by adrenalectomy while that produced by single or successive subcutaneous injection was abolished in adrenalectomized mice. These observations suggest that the decrease in EST following systemic injection of zinc is produced by way of the adrenal gland. However, the mode of action of zinc on the adrenal gland is not clear.

In this paper, the effects of zinc on the adrenal gland with reference to brain excitability are studied and discussed.

MATERIAL AND METHODS

All experiments were performed on male rats of Wistar-strain, 160 to 180 g in body weight. The animals were maintained on a commercial food for labarotory rats known as Oriental Chow. Room temperature was 17–21°C. Zinc, as the chloride, was dissolved in 0.9% NaCl solution

adjusted to pH 2.0 by adding HCl. A dose of 2 mg/kg body weight was of Zinc given subcutaneously. Injection volume was 0.5 ml. Control rats received the same volume of 0.9% NaCl solution (pH 2.0). Adrenalectomy was performed under ether anesthesia through lumbar incision. The animals were sacrificed by ether inhalation.

I) Brain excitability

For determination of brain excitability, the electroshock seizure threshold (EST) was measured using Woodbury and Davenport's[4] apparatus (1952). As the electrical stimulus, 60 cycle/sec. alternating current was applied by means of corneal electrodes for 0.2 sec. EST was defined as the current to produce facial clonus and rhythmic movements of the vibrissae, jaws and ears according to Wachstein et al.[5]

For determination of EST, the current required to evoke the EST seizure in 50% of the animals (EST-50) was calculated by the method of Litchfield and Wilcoxon[6]. To represent changes in EST, EST in control rats was measured and the average was used as the basis for comparison of the experimental values.

II) Adrenal weight

1) Adrenal weight in rats not given unilateral adrenalectomy.

After daily administration of zinc for 10 days, the animals were sacrificed about 12 hours after the last injection and weight of bilateral adrenal glands was taken as quickly as possible.

2) Adrenal weight after unilateral adrenalectomy

The animals were divided into 5 groups. Group I was intact rats. Bilateral adrenal weight was measured. In group II, left adrenal weight was taken 10 days after right adrenalectomy. In group III, a daily dose of 1 mg of cortisone was injected intramusclarly for 10 days after right adrenalectomy. In group IV, cortisone (1 mg, intramuscular) and 0.9% NaCl solution (pH 2.0, Subcutaneous) were injected for 10 days after right adrenalectomy. In group V, cortisone and zinc (2 mg/kg, pH 2.0 subcutaneous) were injected for 10 cays following right adrenalectomy.

The animals in group III, IV and V were sacrificed about 12 hours after the last injection and left adrenal weight was measured.

The increase rate of adrenal weight was calculated according to the following formula of Asanuma:[7]

$$\text{Increase rate of adrenal weight } (\%) = \frac{(\text{left adrenal weight}) - (\text{right adrenal weight})}{(\text{right adrenal weight})} \times 100$$

III) Histochemical studies

The animals were sacrificed about 12 hours after the last adminissration of zinc and the specimens obtained from the adrenal gland were fixed or frozen as quickly as possible. Frozen sections in a cryostat were cut 15 micron thick and paraffin sections were cut 10 micron thick.

Alkaline phosphatase activity was determined by the method of Takamatu and Nishi[8]. Cold acetone paraffin sections were incubated for 10 hours in a solution which consisted of equal volumes of 1% sodium-β-glycerophosphate and calcium chloride borate buffer. Then, they were immersed in 5% silver nitrate solution for 10 minutes.

Acid phosphatase activity was determined by the modification of Gomori's method[9]. Cold acetone paraffin sections were incubated for 3 hours in the solution containing 2% sodium-β-glycerophosphate, 0.2 M acetate buffer (pH 5.0) and 2% lead nitrate. Thereafter, theywere immersed in 1% yellow ammonium sulfate for one minute.

Adenosine triphosphatase (ATP-ase) activity was demonstrated by using the Wachstein and Meisel method[5]. The adrenal gland was fixed in 10% formaline for about 24 hours, and frozen sections were obtained. These sections were incubated for 15 minutes. The following concentration of incubation reagents was used: 20 ml of 0.125% ATP solution, 20 ml of 0.5 M tris-hydrochloride buffer (pH 7.2), 3 ml of 2% lead nitrate solution, 5 ml of 0.1 M magnesium sulfate, 2 ml of water. After that, they were immersed in 1% yellow ammonium sulfidesolution for one minute.

DPN- and TPN- diaphorase, lactic dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PD) were studied using the nitro-BT method[10]. In DPN- and TPN- diaphorases, fresh frozen sections were incubated for 5 minutes. The following concentration of incubation reagents was used: 0.5 ml of 0.1% nitro-BT, 0.5 ml of 0.1 M phosphate buffer (pH 7.2–7.4), 5 mg of DPNH or TPNH and 0.5 ml of distilled water. In LDH and G-6-PD, 0.5 ml of 0.1% nitro-BT, 0.5 ml of 0.1 M tris-malate buffer, 5 mg of DPN or TPN, DL-sodium lactate or glucose-6-phosphate and 0.5 ml of distilled water were used.

Succinic dehydrogenase (SDH) activity was demonstrated by the technique of Nachlas et al.[11] The incubation medium consisted of equal parts of the following two solutions: a; 5 ml of 0.2 M phosphate buffer (pH 7.6) and 5 ml of 0.2 M sodium succinate, b; 10 ml of aqueous solution of nitro-BT (1 mg/ml). Fresh frozen sections were incubated for 20 minutes.

Adrenalin and noradrenalin were studied using the Henle method[12]. The adrenal gland was fixed in Orth-solution for about 48 hours.

Thereafter, paraffin sections were cut. Noradrenalin was demonstrated by using the Hillarp and Hökfelt method[13]. The adrenal gland was fixed in 10% KIO_3 solution for 24 hours and then in 10% formaline for 12 hours. Thereafter, frozen sections were cut.

Ascorbic acid was studied using the Deane and Morse method[14]. Lipid was stained with Sudan-III.

RESULTS

I) The effect of subcutaneously injected zinc on EST in intact and adrenalectomized rats.

As shown in Fig. 1, Subcutaneously injected zinc produced a marked lowering in EST in intact rats. The EST conspicuously decreased as early as 15 minutes after the injection and this effect was found 30 minutes, was also one and a half hours and 6 hours after the injection.

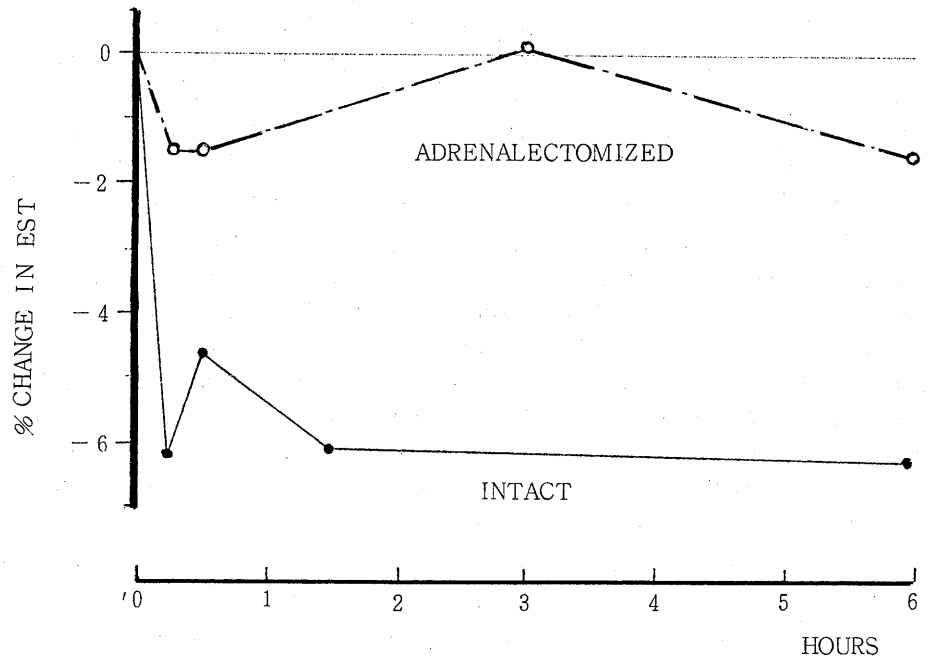


Fig. 1. Effect of a single dose of zinc (2 mg/kg, subcutaneously) on EST in adrenalectomized rats and intact rats.
The change in EST produced by zinc was expressed as % change from the corresponding control values. Nine to 15 rats, weighing 160 to 180 g, were used for each EST-50 determination.

In adrenalectomized rats, the EST-lowering effect of zinc was minimal or insignificant. These experimental results are in accord with Fuchimoto's observation in mice[3].

II) The effect of zinc on adrenal weight.

1) The effect of zinc on adrenal weight in intact rats.

As shown in Fig. 2. zinc markedly increased adrenal weight. In zinc administered rats the average weight was 60.1 ± 2.5 mg (mean ± standard error) with arange of 52.4 to 68.0 mg, while in control rats it was 40.2 ± 1.5 mg with the range of 35.0 to 46.8 mg. This difference is statistically significant at the 0.5% level.

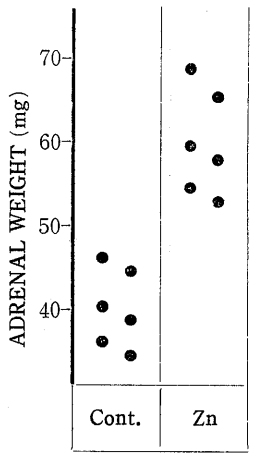


Fig. 2. Effect of zinc on adrenal weight

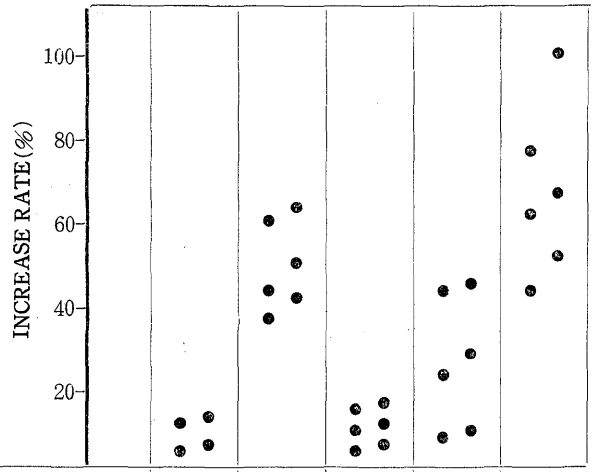
Cont: Control group was given 0.9% NaCl solution (pH 2.0, 0.5 ml) subcutaneously once daily for 10 days.

Zn: Zinc group was given zinc (2 mg/kg, pH 2.0, 0.5 ml) subcutaneously once daily for 10 days. The rats were sacrificed by ether inhalation about 12 hours after the last administration and weight of bi lateral adrenal glands was taken. Six animals were used in each group.

2) The effect of zinc on weight of the contralateral adrenal gland after unilateral adrenalectomy.

Experimental results in this series are summarized in Fig. 3. After right adrenalectomy, the increase rate of the left adrenal weight was 49.0 ± 4.8% as shown in group II, but it decreased to 10.8 ± 2.1% by cortisone injection as shown in group III. This indicates that the hypertrophy of the left adrenal gland after right adrenalectomy is completely suppressed by the administration of a daily dose of 1 mg of cortisone, because the left

adrenal gland was 9.5 ± 1.7% heavier than the right adrenal gland in intact rats (group I). These results are almost in accord with the experimental results of Asanuma[7]. In the rats which were administered cortisone for 10 days after right adrenalectomy, zinc markedly increased the weight of the left adrenal gland, the increase rate was 69.0 ± 8.0% as shown in group V, being markedly higher than the increase rate of 27.0 ± 7.5% in the control group (group IV). This difference is statistically significant at the 0.5% level.



Group	I	II	III	IV	V
Unilateral adrenalectomy	(−)	(+)	(+)	(+)	(+)
Cortisone (1 mg)	(−)	(−)	(+)	(+)	(+)
0.9% MaCl solution	(−)	(−)	−)	(+)	(−)
Zinc (2 mg/kg)	(−)	(−)	(−)	(−)	(+)

Fig. 3. Effect of zinc on weight of the contralateral adrenal gland after unilateral adrenalectomy

The increase rate of adrenal weight was calculated by the method of Asanuma[1]. Group I: no treatment and no administration. Group II: unilateral adrenalectomy alone. Group III: unilateral adrenalectomy plus administration of cortison. Group IV: unilateral adre nalectomy plus administration of cortison and 0.9% Nacl solution. Group V: unilateral adrenalectomy plus administration of cortison and zinc.

III) Histochemical changes of the adrenal gland following the administration of zinc.

Histochemical changes in zinc administered rats compared with those in control rats are summarized in Table 1.

Table. 1. Histochemical findings in the four parts of the adrenal gland.

	Medulla		Cortex					
			Z. reticul.		Z. fascicul.		Z. glomerul.	
	C	Zn	C	Zn	C	Zn	C	Zn
Alkaline phosphatase	(−)	(−)	(−)	(卅)	(+)	(卅)	(+)	(卅)
Acid phosphatase	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
ATP-ase	(+)	(+)	(+)	(卅)	(+)	(卅)	(+)	(卄)
G-6-PD	(+)	(+)	(卄)	(卄)	(卄)	(卄)	(卄)	(卄)
SDH	(−)	(−)	(卅)	(卅)	(卄)	(卅)	(卄)	(卄)
LDH	(−)	(−)	(卄)	(卄)	(卄)	(卄)	(卄)	(卄)
TPN-diaphorase	(−)	(−)	(卄)	(卄)	(卄)	(卄)	(卄)	(卄)
DPN-diaphorase	(−)	(−)	(卄)	(卄)	(卄)	(卄)	(卄)	(卄)
Norad. (卄) Adrenaline	(卄)	(卄)	(−)	(−)	(−)	(−)	(−)	(−)
Noradrenaline	(卄)	(卄)	(−)	(−)	(−)	(−)	(−)	(−)
Ascorbic acid	(−)	(−)	(+)	(+)	(卄)	(卄)	(+)	(+)
Lipid	(−)	(−)	(卄)	(卄)	(卅)	(卅)	(卄)	(卄)

A daily dose of 2 mg/kg body weight of zinc was given subcutaneously for 10 days.
C: control Zn: zinc administration (−): no staining (+): weak staining
(卄): moderate staining (卅): heavy staining

In zinc administered rats, alkaline phosphatase activity in the capillary wall including reticuloendothelial cells markedly increased in the reticular, fascicular and glomerular layers, although in control ratsthis activity was found slightly only in the fascicular layer.

ATP-ase activity was much the same as alkaline phosphatase activity; it markedly increased in the capillary wall including reticuloendothelial cells in the three layers by zinc administration, while it was weak in the controls.

SDH activity in zinc administered rats slightly increased in the fascicular layer compared with that in the controls.

On the other hand, the activities of acid phosphatase, G-6-PD, LDH, TPN-diaphorase and DPN-diaphorase did not show any significant changes between zinc administered rats and control rats.

Adrenalin plus noradrenalin end noradrenalin in the adrenal medulla were not changed by zinc administration.

In unilaterally adrenalectomized rats (group II), histochemical findings of the contralateral adrenal gland were as follows: slight acceleration of SDH activity was nearly the same but alkaline phosphatase and ATP-ase activities were lower in comparison with the findings of zinc administered rats.

In ascorbic acid and lipid, there were no changes except a slight increase of lipid granules in the fascicular layer.

DISCUSSION

A single subcutaneous injection of zinc markedly decreased EST in intact rats but this EST-lowering effect was lost in adrenalectomized rats. In regard to the relation between the adrenal gland and brain excitability, Woodbury (1954, 1958)[15),16)] reportd several experimental findings in rats. The EST remarkably decreased after adrenalectomy or several glucocorticoids injections, while mineralocorticoid (DCA) markedly increased and ACTH slightly increased EST. On the other hand, Swinyard (1962)[17)] has shown inmice that epinephrine causes an increase in brain excitability which occurs almost instantly. Therefore, it is quite possible that the EST-lowering effect of zinc is produced by way of the adrenal gland.

Many studies on the relations between the function and the structure of the adrenal gland have been reported. Especially, it is thought that the increment of adrenal weight shows a stimulation of adrenal function. Sayers (1950)[18)], Tepperman (1948)[19)], Mori (1955)[20)] and other investigators support this. A remarkable increase in adrenal weight produced by the administration of zinc in the present study indicates the stimulation of the adrenal gland by zinc.

Concerning ascorbic acid, Sayers et al. (1945, 1947)[21),22)] found that the concentration of ascorbic acid in the adrenal gland was more than that in other organs, and that it decreased when ACTH acted on the adrenal gland. Deane (1948)[14)] also, in ACTH administered rats, demonstrated a remarkable decrease of ascorbic acid concentration in the fascicular and reticular layers.

In relation to lipid, Deane (1948)[23)] has described from the histological viewpoints, that lipid granules in the adrenal cortex increase and are coarse in adrenal hypofunction, while they decrease and are fine in its hyperfunction.

In the present study, ascorbic acid and lipid are not significantly changed by zinc administration. Therefore, the changes in ascorbic acid and lipid of the adrenal gland produced by the administration of zinc are

different from those produced by the administration of ACTH.

Recent progress in enzymohistochemistry provides valuable clues to the study on functional changes of many organs.

Dawson et al. (1961)[24] showed in intact rats that alkaline phosphatase, acid phosphatase, LDH, SDH, non specific esterase, DPN-diaphorase and lipid distribute in each layer of the adrenal cortex and the adrenal medulla, and these six enzymes and lipid were not significantly changed in ACTH administered rats. Misutani (1961)[25] and McKerne (1964)[26] reported that ATP-ase, G-6-PD, LDH and TPN-diaphorase were heavily stained in the adrenal gland of rats.

Meusers (1966)[27] demonstrated that the activities of G-6-PD, SDH, ATP-ase and alkaline phosphatase in the adrenal gland decreased in cortisone administered rats. Glick (1958)[28] described that the SDH activity of the adrenal gland was moderatly accelerated in ACTH administered rats. Sasano (1966)[29] described how the adrenal gland was stimulated by glycyrrhizin from several examinations. In his enzymohistochemical study on acid phosphatase, alkaline phosphatase, ATP-ase, G-6-PD, SDH, TPN- and DPN-disphorase, the ATP-ase and alkaline phosphatase activities of the adrenal gland were slightly accelerated but other enzyme activities were not significantly changed in ACTH administered rats. In glycyrrhizin administered rats, a moderate acceleration of ATP-ase and alkaline phosphatase activities was found.

Considering these reports on enzymohistochemical studies, it seems that the acceleration of ATP-ase, alkaline phosphatase and SDH activities is found in hyperfunction of the adrenal cortex and the suppression of ATP-ase, alkaline phosphatase, G-6-PD, and SDH activities is found in hypofunction.

In the present study, after unilateral adrenalectomy, the contralateral adrenal gland showed compensatory hypertrophy due to the elevation of ACTH excretion. In zinc administration, SDH activity was nearly the same but alkaline phosphatase and ATP-ase activities were much higher in comparison with the enzymohistochemical findings of the contralateral compensatory hypertrophic adrenal gland. Therefore, the effect of zinc on the adrenal gland can not be explained by the elevation of ACTH excretion alone.

It has been described by several investigators (Yagawa, Akasaki)[30,31] from the histological viewpoint, that the capillary wall of the adrenal gland contains reticuloendothelial cells comparable to Kupper's cells in the liver. On the other hand, Berliner et al. (1964)[32] have demonstrated in the bovine adrenal gland that iron-containing cell (reticuloendothelia cell) and no

iron-containing cell (parenchymal cell) can be successfully separated by means of a magnetic technique, and that the adrenal reticuloendothelial cells produce more cortisol from progesterone than parenchymal cells. do it.

In the present study, the most remarkable finding is the marked acceleration of ATP-ase and alkaline phosphatase activities in the capillary wall including the reticuloendothelial cells in the reticular and fascicular layers in zinc administrated rats. From these findings, it seems tobe evident that zinc stimulates the adrenal gland itself. Probably the subcutaneously injected zinc rapidly enters the blood stream and stimulates the capillary wall including the reticuloendothelial cells in the adrenal cortex. And then, the reticuloendothelial cells stimulated by zinc bring about an increase in the excretion of glucocorticoids, the mechanism of which is supported by the study of Berliner[32]. Because glucocorticoids have been shown to decrease EST markedly, it is reasonable that the EST-lowering effect of zinc is produced by the increment of glucocorticoid level.

On the other hand, from the findings that glycyrrhizin did not change adrenal weight significantly in intact rat but releasesthe inhibition of cortisone on the compensatory hypertrophy of the contralateral adrenal gland after unilateral adrenalectomy and mcreases adrenal weight, it is thought that glycyrrhizin inhibits the negative feedback control in the pituitary-adrenal system in cortisone administered (Asanuma, Sasano)[7),29)].

Because zinc markedly increased adrenal weight in both intact rats and unilateral adrenalectomized rats to whom cortisone was administered, zinc seemed not only to inhibit the negative feedback control but to stimulate the adrenal cortex directly. Millar's observation (1961)[33] on rats that a high ^{65}Zu concentration was shown in the adrenal cortex also supports this assumption.

In the adrenal medulla, Allen (1956)[34] histochemically demonstrated the loss of adrenaline, noradrenaline and a slight increment of alkaline phosphatase activity in the adrenal medulla of mice following cold stress at 4°C for 7 days and the same histochemical changes were found during inanition. These findings indicate that changes in adrenalin and noradrenalin are easily detectable in histochemical study. In the present study, adrenaline, noradrenaline and alkaline phosphatase activities were not significantly changed by zinc administration. Therefore, it is unlikely that zinc acts on the adrenal medulla.

Judging from the above findings and discussion, it may be concluded that the increase in brain excitability produced by zinc is caused by the hyperfunction of the adrenal cortex, mostly due to the direct action of zinc on this part of the organ.

SUMMARY

1. The effect of zinc on the adrenal gland with special reference to the increasing effect on brain excitability was studied in rats.

2. A single subcutaneous injection of zinc markedly decreased EST. This EST-lowering effect was lost in adrenalectomized rats.

3. Succesive subcutaneous injections of zinc markedly increased adrenal weight in intact rats and the weight of the contralateral adrenal gland inhibited by cortisone after unilateral adrenalectomy.

4. The most remarkable change in histochemical examination produced by zinc administration was a marked acceleration of ATP-ase and alkaline phosphatase activities in the capillary wall including the reticuloendothelial cells in the adrenal cortex.

5. The histochemical findings of the adrenal medulla were not significantly changed by zinc administration.

6. From the above findings, it is concluded that the EST-lowering effect of systemically administered zinc is produced by the stimultibon of the adrenal cortex. Its possiale mechanism is discussed.

REFERENCES

1) Tokuoka, S.: Patho-physiological studies on epilepsy. *Acta School. Med. Univ. Kiottn,* **29** : 15-30, 1951
2) Peters, H. A.: Porphyric psychosis and chelation therapy. *Recent Advances in Biological Psychiatry,* **4** : 204-217, 1962
3) Fuchimoto, T., Watanabe, M., Fujii, M., Takashima, M. and Hiraoka, H.: The effect of zinc on the central nervous system. *Bull. Yamaguchi Med. School,* **14** : 67-78, 1967
4) Woodbury, L. A. and Davenport, V. D.: Design and use of a new electroschock seizure appartus, and analysis of factors altering seizure threshold and pattern. *Arch. Int. Pharmacodyn.,* **92** : 92-107, 1952
5) Wachstein, M. and Meisel, E.: Histochemistry of hepatic phosphatases at a physiologic pH with special reference to the demonstration of bile can aliculi. *Amer. J. Clin, Pathol.,* **27** : 13-23, 1957
6) Litchfield, J. T. Jr., and Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. *J. Pharmacol.,* **96** : 99-113, 1949
7) Asanuma, Y.: Effect of glyzyrrhizin on the suppressive action of glucocorticoid on the pituitary adrenal axis. *Folia Endocrinol. Jap.,* **41** : 623-632, 1965
8) Takamatsu, H. and Nishi, H.: Histochemical study of phosp hatase. *Folia Pathol. Jap.,* **43** : 546-551, 1954
9) Gomori, G.: Histochemical methods for acid phosphatase. *J. Histochem. Cytochem.,* **4** : 453-461, 1956
10) Pearse, A. G. E.: *Histochemistry. Theoretical and Applied.* 2nd ed., Churchill, London, 1961
11) Nachlass, M. M., Walker, D. G. and Seligman, A. M.: A histochemical method for the demonstration of diphosphophridine nucleotid diaphorase. *J. Biophys. Biochem. Cytol.,* **4** : 29-38, 1958

12) Henle, J.: *Z. Ration. Med.*, 24 : 143, 1865 (Microscopic Histochemistry. 3rd ed., Igaku-Shoin, Tokyo, 1965)
13) Hillarp, N-A. and Hökfelt, B.: Histochemical demonstration of noradrenaline and adrenaline in the adrenal medulla. *J. Histochem. Cytochem.*, 3 : 1-5, 1955
14) Deane, H. W. and Morse, A.: The cytological distribution of ascorbic acid in the adrenal cortex of the rat under normal and experimental conditions. *Anat. Rec.*, 100 : 127-141, 1948
15) Woodbury, D. M.: Relation between the adrenal cortex and the central nervous system. *Pharmacol. Rev.*, 10 : 275-357, 1958
16) Woodbury, D. M.: Effect of hormones on brain excitability and electrolytes. *Recent Progr. Hormone Res.*, 10 : 65-107, 1954
17) Swinyard, E. A., Radhakrishnan, N. and Goodman, L. S.: Effect of brief restraint on the convulsive threshold of mice. *J. Pharmacol. Exp. Therap.*, 138 : 337-342, 1962
18) Sayer, G.: The adrenal cortex and homeostasis. *Physiol. Rev.*, 30 : 241-320, 1950
19) Tepperman, J. and Bogardus, J. S.: The functional morphology of the adrenal cortex. *Endocrinol.*, 43 : 448-454, 1948
20) Mori, S.: *Naibunpitu-no-Tudoi*, 7 : 2-4, 1955 (Japanese)
21) Sayers, G. and Sayers, M. A.: Regulation of pituitary adreocorticotropic activity during the response of rat to acute stress. *Endocrinol.*, 40 : 265-272, 1947
22) Sayers, G., Sayers, M. A., Liang, T. Y. and Long, C. N. H.: The cholesterol and ascorbic acid content of adrenal liver and plasma following hemorrhage. *Endocrinol.*, 37 : 96-107, 1945
23) Deane, H. W., Shaw, J. H. and Greep, R. O.: The distribution of lipid in the adrenal cortex under experimental conditions. *Endocrinol.*, 43 : 133-151, 1948
24) Dawson, I. M. P., Pryse-Davies, J. and Snape, I. M.: The distribution of six enzyme systems and lipid in the human and rat adrenal cortex before and after administration of steriod and ACTH. *J. Path. Bact.*, 81 : 181-195, 1961
25) Misutani, A. and Hikima, K.: Oxidative enzymes in paraffin sections. *Acta Tubercul. Jap.*, 11 : 17-27, 1961
26) Mckerns, K. W.: Mechanism of action of ACTH through activation of glucose-6-phosphate dehydrogenase. *Acta Biochem. Biophys.*, 90 : 375-371, 1964
27) Meusers, P. J.: Fermenttopochemische Untersuchungen an der Nebennierenrinde des Meerschweinchens nach Abschlusseiner langfristigen Cortisonvorbehandlung. *Histochemie*, 7 : 50-63, 1966
28) Glick, D. and Greenberg, L. J.: The quantitative histological distribution of the succinic dehyrogenase system in rat adrenal and the influence of hypophysectomy, ACTH, and cortisone. *Endocrinol.*, 63 : 778-784, 1958
29) Sasano, N., Miyazawa, H., Shimizu, K. and Koizumi, K.: Stimulation of rat adrenocortical cells by glycyrrhizin with special reference to its inhibitory effect on dexamethasone-induced atrophy of the adrenal cortex. *Tohoku J. Exp. Med.*, 90 : 391-403, 1966
30) Yagawa, K.: Study on the reticuloendothelial system of the suprarenals. *Niigata Igaku Zasshi*, 64 : 556-564, 1950 (Japanese)
31) Akazaki, K.: A concept of reticuloendothlial system. *Tohoku J. Exp. Med.*, 76 : 107-118, 1962
32) Berliner, D. L., Nabors, C. G. and Dougherty, T. F.: The role of hepatic and adrenal reticuloendothelial cells in biotransformation. *J. Reticuloendothel. Soc.*, 1 : 1-17, 1964
33) Millar, M. J., Vincent, N. R. and Maswon, C. A.: An autoradiographic study of the distribution of zinc-65 in rat tissue. *J. Histochem. Cytochem.*, 9 : 111-116, 1961
34) Allen, J. M.: The influence of cold inantion and insulin shock upon the histochemistry of the adrenal medulla of the mouse. *J. Histochem. Cytochem.*, 4 : 341-346, 1956