dine[19] and 10.0 g. of MeI was warmed in MeOH on a water bath for 3 hr., allowed to stand at room temperature over night, and filtered to collect the methiodide as pale yellow crystals (from MeOH), m.p. 242° (decomp.). Yield, 0.93 g.

The methiodide was converted to the chloride by the usual procedure and its EtOH solution was submitted to catalytic reduction over PtO_2. The catalyst was filtered off, EtOH was evaporated from the filtrate, and the residue was dissolved in H_2O. This solution was basified with Na_2CO_3, salted out with K_2CO_3, and extracted with Et_2O. After drying over K_2CO_3, Et_2O extract was evaporated and the residue was distilled in a reduced pressure to furnish colorless oil, $b.p_{10}$ 135°. Yield, 0.53 g. (54%).

Dipicrate: Yellow crystals, m.p. 208~209° (from EtOH). *Anal.* Calcd. for $C_{15}H_{30}N_2 \cdot 2C_6H_3N_3O_7$: C, 46.55; H, 5.17; N, 16.09. Found: C, 46.20; H, 5.26; N, 15.73. Au-salt: Yellow crystals, m.p. 210° (decomp.). *Anal.* Calcd. for $C_{15}H_{30}N_2 \cdot 2HAuCl_4$: C, 19.65; H, 3.49; Au, 42.85. Found: C, 19.58; H, 3.37; Au, 42.49.

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Summary

3-Butyl-, 3-pentyl-, 3-isopentyl-, and 3-phenethylquinolizidines were found to have a comparatively strong uterus-contracting action like that of sparteine.

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Sumiyuki Akihama and Shigeshi Toyoshima: Antiviral Effect of Zinc Complexes on Japanese B Encephalitis Virus.*[1]

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Ueda and Toyoshima[1] reported that *erythro*-1-(*p*-tolyl)-2-aminopropanol methansulfonate named "Methodrine" exerted a therapeutic effect on Japanese B encephalitis in mice. Totani[2] also found that a curative effect on patients suffering from Japanese B encephalitis (90% of patients were completly cured) was obtained by the simultaneous administration of this drug and ACTH-Zn, and asserted that such a synergistic action was not observed with other adrenocortical steroid drugs and ACTH preparation containing no zinc. These findings suggested that zinc itself might possess an antiviral effect or potentiate the effect of other drugs on the virus. This paper describes a survey of the antiviral effect of zinc complexes of several amino acids and organic reagents on Japanese B encephalitis virus in mice.

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Syntheses of Zinc Complexes

Although many of zinc complexes have been reported to date, there are few which were isolated as crystalline compounds. Zinc complexes used in this antiviral experiment were prepared as follows. Since zinc complexes of glycine,[3] 8-hydroxyquinoline,[4] diethyldithiocarbamic acid,[5] diphenylthiocarbazone,[6] 3,4-dimercaptotoluene,[7] and 1,10-phenanthroline-3,4-dimercaptotoluene,[8] were already reported by several researchers, they were synthesized according to the methods described in those papers. Other complexes having a general formula of $Ke_m \cdot Zn \cdot nH_2O$ were prepared by allowing to react ligands with zinc oxide or zinc hydroxide, or with zinc chloride followed by neutralization of the resulting products with alkali hydroxide solution. Complexes having a general formula of $Ke_m \cdot ZnCl_2 \cdot nH_2O$ were also obtained by treating ligands or their hydrochlorides with zinc chloride. Here, *m* and *n* indicate positive integers. In these reactions, crude zinc complexes were, in general, precipitated from the reaction mixtures, and purified by recrystallization from appropriate solvents. The zinc complexes prepared are listed in Table I.

Table I.

Compound	Mol. Formula	m.p. (°C)	Yield (%)	Method	N%		Zn%	
					Calcd.	Found	Calcd.	Found
DL-Alanine-Zn	$(C_3H_6O_2N)_2Zn$	335~336	78	a	11.60	11.72	27.06	27.79
L-Leucine-Zn	$(C_6H_{12}O_2N)_2Zn$	324~325	55	a	8.60	8.49	20.07	20.50
DL-Methionine-Zn	$(C_5H_{10}O_2NS)_2Zn$	>360	75	a	7.77	7.82	18.13	17.93
L-Cysteine-Zn	$C_3H_5O_2NS \cdot Zn$	>360	84	b	7.59	7.29	32.26	31.61
DL-Lysine-Zn	$(C_6H_{13}O_2N_2)_2Zn \cdot 6H_2O$	227~228	47	a	12.08	12.00	14.09	14.39
DL-Phenylalanine-Zn	$(C_9H_{10}O_2N)_2Zn$	291~292	55	b	7.12	7.14	16.60	17.10
DL-Asparagine-Zn	$(C_4H_7O_3N_2)_2Zn$	>360	63	a	17.10	17.18	19.95	19.84
DL-Aspartic acid-Zn	$C_4H_5O_4N \cdot Zn \cdot H_2O$	>360	69	a	6.53	6.52	30.41	30.51
L-Tyrosine-Zn	$(C_9H_{10}O_3N)_2Zn \cdot 4H_2O$	279	43	a	6.93	6.69	8.09	7.87
2-Picolinic acid-Zn	$(C_6H_4O_2N)_2Zn \cdot 3H_2O$	102~103	82	a	7.70	7.70	17.97	17.81
Guanidine-Zn	$(CH_5N_3)_2ZnCl_2 \cdot 4H_2O$	176~177	86	c	25.74	26.13	20.02	20.23
1,10-Phenanthroline-Zn	$C_{12}H_8N_2 \cdot ZnCl_2$	>360	84	d	8.85	8.23	20.66	21.15

a) Determined by using EDTA titration after decomposition with nitric acid and sulfonic acid.[9]

Screening Test with Zinc Complexes

The primary *in vivo* screening test as to the antiviral effect of zinc complexes were carried out by inoculating 10^{-2} virus dilution of the Nakayama strain of Japanese B encephalitis virus ($LD_{50}=10^{-2}$ in intraperitoneal route) intraperitoneally into mice according to the method described in the experimental part. The results are shown in Table II, from which it is seen that χ^2 values of the zinc complexes of asparagine and 1,10-phenanthroline 3,4-dimercaptotoluene show 3.14 and 4.18, respectively. These values are not considered very remarkable, compared with those of the antiviral compounds previously reported by the authors group. However, it may be said that both asparagine and 1,10-phenanthroline-3,4-dimercaptotoluene zinc complexes were fairly effective on the infection of the Nakayama strain in mice among the zinc complexes tested.

The secondary tests of these two compounds were conducted by using the Nakayama strain. The experimental procedures were the same as described in the experimental

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Table II. Antiviral Activities against Japanese B encephalitis Virus

Compound	Dose (mg./kg.)	Treated group	Untreated group	$\chi^{2b)}$
Glycine-Zn	25	8/10[a)]	7/11[a)]	—
DL-Phenylalanine-Zn	125	6/10	7/11	—
L-Leucine-Zn	58	6/10	7/11	—
DL-Aspartic acid-Zn	25	4/10	7/11	1.17
DL-Asparagine-Zn	25	2/11	6/11	3.14
DL-Alanine-Zn	92	7/11	6/11	—
L-Cysteine-Zn	25	4/11	6/11	—
DL-Lysine-Zn	182	7/11	6/11	—
L-Tyrosine-Zn	250	5/10	6/10	—
DL-Methionine-Zn	125	8/10	6/10	—
2-Picolinic acid-Zn	50	5/11	9/20	—
Guanidine-Zn	25	6/11	9/20	—
Diethyl dithiocarbamic acid-Zn	13	8/11	9/20	—
Diphenylthiocarbazone-Zn	292	8/14	9/20	—
8-Hydroxyquinoline-Zn	150	4/11	9/20	—
3,4-Dimercaptotoluene-Zn	500	5/9	9/20	—
1,10-Phenanthroline-Zn	25	4/10	9/20	—
1,10-Phenanthroline-3,4-dimercaptotoluene-Zn	300	1/11	9/20	4.18
ACTH-Zn[c)]	(8 unit/kg.)	10/15	13/15	1.67
Protamine-Zn-Insulin[c)]	(10^{-3} unit 0.1 cc./10 g.)	4/11	6/11	0.73

a) Number of the mice showed paralysis/ the total mice used.
b) $P(\chi^2\ 3.84)=0.05$
c) Commercial preparation.

Table III. The *in vivo* Screening Test of Zinc Complexes on the Nakayama Strain of Japanese B encephalitis Virus

Virus dilution	Asparagine-Zn	1,10-Phenanthroline-3,4-dimercaptotoluene-Zn
$10^{-1.5}$	28/29 (26/29)[a)]	28/31 (26/29)
10^{-2}	2/11 (6/11)	1/11 (9/20)

a) Figures in the parentheses represent the number of control group.

part, except for the viral inoculum size, and $10^{-1.5}$ dilution of the viral material was employed.

The experimental results shown in Table III clarified that both the complexes were ineffective on the virus. Therefore, it may be concluded that they were effective on the virus in comparatively lower concentrations, which nearly correspond to LD_{50} of the virus in mice.

Next, to clarify whether this curative effect is due to the virus inactivating action or not, asparagine zinc complex was tested by using the method described in the experimental part. As shown in Table IV, the complex did not show any viral inactivating effect against the virus.

Table IV. Virus Inactivation Action of Asparagine Zinc Complex

Treated group with asparagine-Zn		Control group.
0.05%	10^{-6}	$10^{-7.8}$
0.025%	10^{-6}	$10^{-7.8}$
0.01%	10^{-6}	$10^{-7.8}$

This fact suggests that the antiviral effect of asparagine zinc complex should not be ascribed to a virus inactivating action. Accordingly, it may be inferred that the complex might produce a curative effect through influence upon the intracellular multiplication of the virus, rather than a direct virocidal action.

It is of interest to investigate the relationship between the complexity and antiviral

effect of zinc complexes. However, effective asparagine zinc complex has a stability constant (log K) of 8.7, while the other ineffective complexes possess constants between 7.3 and 20.9.[10] Therefore, at present, there can be found no functional relation between stability constants and antiviral activities of zinc complexes.

On the other hand, it may be considered that ligands in the zinc complexes might play a more important role than simple zinc ion concentration in the development of antiviral activity.

This assumption leds to the possibility of finding more effective zinc complexes by replacing asparagine with ligands other than those employed in these studies.

The work on this is now in progress, and the results will be published in the future.

Experimental

General Procedure for Preparation of Zinc Complexes——a) To a solution of 0.02 mole of ligands in 20 cc. H_2O, 0.01 mole of ZnO or $Zn(OH)_2$ was added with stirring and the mixture warmed on a steam bath for 2 hr. at 40~50°. The reaction mixture was treated with 60 cc. of EtOH and allowed to stand. The separated crystals was filtered off, and recrystatallized from hydr. EtOH.
b) After 0.02 mole of ligands and 0.01 mole of $ZnCl_2$ were dissolved in 20 cc. of dil. HCl, the mixture was neutralized with dil. NH_4OH. The produced precipitate was filtered off, and washed with H_2O and EtOH.
c) To an aqueous solution of 0.05 mole of the hydrochlorides of ligands, 0.025 mole of $ZnCl_2$ was added, and the mixture evaporated on a steam bath. The residue was crystallized from hot H_2O.
d) To a solution of 0.01 mole of ligands in 30 cc. of EtOH, 0.005 mole of $ZnCl_2$ was added with stirring. After that, separated crystals were filtered off, and recrystallized from hydr. EtOH.

Test of Antiviral Activity.

1) Materials : The Nakayama strain of Japanese B encephalitis virus was supplied by the courtesy of Dr. S. Kasahara, Director of Kitasato Institute.

Mice of D. M. K. strain of 8~10 g. in body-weight were used for the experiments.

2) Method of Screening Test : 0.3 cc. of 10^{-2} virus dilution of the Nakayama strain virus (LD_{50} $=10^{-2}$ in intraperitoneal route) was inoculated intraperitoneally into mice, and 72 hr. later, 1/3 LD_{50} of each of zinc complexes tested was injected intraperitoneally into these mice with a single dose. After the daily observation for two weeks, the ratio of the number of the mice which showed paralysis to the total mice used was recorded.

3) Method for Test of Virus Inactivating Action : Various dilutions of the Nakayama strain were prepared, and then 0.1 cc. of each of the dilutions was added into test tubes containing 0.1 cc. of three different dilutions of asparagine zinc complex. After these mixtures were kept for 1 hr. at room temperature, 0.1 cc. of each of these mixture solutions was added into test tubes containing 0.9 cc. of YLA medium supplemented with 5% bovine serum, in which the monolayer sheet of Hela cells had been established. These mixtures were incubated for 72 hr. at 37° and then, for the determination of LD_{50}, 0.03 cc. of the culture fluid of these tubes was inoculated intracerebrally into groups of mice.

Summary

Zinc complexes of glycine, DL-alanine, L-cysteine, DL-phenylalanine, L-leucine, DL-lysine, L-tyrosine, DL-methionine, DL-asparagine, DL-aspartic acid, 2-picolinic acid, 8-hydroxyquinoline, guanidine, diethyldithiocarbamic acid, diphenylthiocarbazone, 3,4-dimercaptotoluene, 1,10-phenanthroline, 1,10-phenananthroline-3,4-dimercaptotoluene and corticotropin, and protamine zinc insulin were prepared and their antiviral activites were examined.

Among these, the zinc complexes of both asparagine and 1,10-phenanthroline-3,4-dimercaptotoluene were found to be fairly effective on the Nakayama strain of Japanese B encephalitis virus *in vivo*.

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