at lower cortisone dosage with the Ak4R than with the parent Ak4 strain, and demonstrates the lack of cross resistance to cortisone in this amethopterin-fast strain (Ak4R) of mouse leukemia.

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Observations on Antiviral Activity of Viscosin.*† (19071)

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A culture of *Pseudomonas viscosa* was isolated by Kochi in the laboratories of the Yokohama Medical College during a systematic search for microorganisms producing antibiotic substances(1). This culture was found to produce an antibiotic which was active *in vitro* against various pathogenic and saprophytic mycobacteria but which was inactive against representative species of other groups of bacteria. This antibiotic, provisionally designated "P-preparation," was obtained in crystalline form and was found to be heat-stable and soluble in ethanol, methanol, ether, acetone, and alkaline phosphate buffer. Taro Miuri(2) reported that the crystalline antibiotic was found to be an acidic polypeptide having a melting point of 264-268°C (Corr.). In preliminary tests this substance was found to exert a demonstrable therapeutic effect on experimental tuberculosis in guinea pigs(1,3) which was minimal when compared to that of streptomycin(3).

A quantity of the crystalline substance was prepared in this laboratory, and the antibiotic was named viscosin(4). In addition to its antibiotic activity against mycobacteria, viscosin was reported to exert a protective effect in embryonated eggs infected with infectious bronchitis virus of chickens and to exert a slight but detectable suppressive effect on the progress of infection in mice infected with influenza A virus(4). A report of the studies on the antiviral properties of viscosin is presented here.

Methods. The following viruses were used in this study: Vaccinia virus, New York City Board of Health strain; Newcastle disease virus, California strain; influenza A virus, PR-8 strain; influenza B virus, Lee strain; infectious bronchitis virus of chickens, original strain isolated by Beaudette and Hudson; and *Miyagawanella felis,* original strain of feline pneumonitis virus isolated by Baker. Inoculum pools of the various viruses containing 30 to 50% yolk or inactivated normal horse serum were prepared, distributed into suitable ampules, and stored at —70°C. In each experiment the infectivity titers of the various viral inocula were determined in the usual manner by inoculation of serial decimal dilutions of the respective pools into groups of 6-10 eggs each. The contact test, a combined *in vitro* and *in vivo* test was performed as follows: Equal volumes of solutions of various concentrations of the antibiotic and appropriate dilutions of virus were mixed *in vitro* and incubated at room temperature for 2 hours. One ml amounts of the various antibiotic-virus mixtures were then inoculated

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into groups of 6 to 10 embryonated eggs each. Two *in vivo* embryo tests were used. In the embryo protection test, the antibiotic was injected one hour prior to infection of the embryo. In the embryo therapeutic test, the antibiotic was injected following infection of the embryo as indicated in the text. For vaccinia virus(5) and *Miyagawanella felis* the criterion of antiviral activity was survival of the embryo following inoculation into the yolk sac of eggs in the 6th day of embryonic development. The influenza viruses were inoculated into the allantoic sac of eggs in the 10th day of embryonic development, and the absence or marked reduction of hemagglutinin in allantoic fluid collected from viable embryos 48 hours after inoculation was used as the criterion for antiviral activity. Infectious bronchitis virus and Newcastle disease virus were also inoculated into the allantoic sac but the criterion of antiviral activity was survival of the embryo. All eggs were incubated at 36°C and candled daily through the 16th day of embryonic development. Hemagglutination tests were performed in the usual manner(6).

Production of viscosin. The original procedure described by Kochi(1,4) for the production of viscosin was used with minor modifications. The culture medium had the following composition: Glycerol, 20 ml; tryptone (Difco), 5 g; beef extract (Difco), 5 g; yeast extract, 2 g; asparagin, 1 g; KH_2PO_4, 5 g; Mg SO_4, .25 g; distilled water to 1000 ml. The medium was adjusted to pH 7.0 before sterilization in the autoclave. Five hundred ml amounts of medium in Blake bottles were seeded with *Pseudomonas viscosa* 122§ and incubated in the horizontal position at 20°C. Maximum antibiotic activity was obtained after 7 to 10 days' incubation when the pH of the culture was 8.5. The broth at that time contained 100 to 200 dilution units per ml against *Mycobacterium tuberculosis* 607 using the streak dilution method(7). The culture was clarified by centrifugation, the broth adjusted to pH 6, and 2% charcoal (Darco G-60) was added. The mixture was agitated for one hour before filtration. The charcoal was then eluted with a volume of acetone equivalent to 30% of the original volume of broth. The acetone was concentrated under reduced pressure until the viscosin flocculated. Precipitation was completed by storing the concentrated mixture for 1 to 3 days at 4°C. Recrystallization was carried out by dissolving the dried precipitate in 100 times its volume in 50% ethanol at 60°C followed by storage in the refrigerator. The yield of crystalline viscosin thus obtained varied from 200 to 600 mg per liter of culture medium. Solutions of viscosin were made in phosphate buffer at pH 7.3 and sterilized by heating to 100°C for 10 minutes.

Experimental. Viscosin was tested for antiviral activity against 6 viral agents by means of the contact test (see *Methods*). The results are summarized in Table I. It will be seen that infectious bronchitis virus was markedly inhibited or inactivated by viscosin. Slight inhibition or inactivation of the influenza viruses and Newcastle disease virus was also apparent. Viscosin was inactive against vaccinia virus and *Miyagawanella felis* in the concentrations tested. In chicken embryos the maximum tolerated dose (LDo) was approximately 0.5 mg via the allantoic sac and greater than 2.0 mg via the yolk sac. However, the toxicity for eggs varied considerably among groups of embryos infected with the various viruses.

The protective and therapeutic effect of viscosin on infectious bronchitis virus in chicken embryos was determined in the following experiment. Various amounts of viscosin were injected into the allantoic sac of embryonated eggs one hour before, one hour after, and 4 hours after infection, respectively, with approximately 100 LD_{50} of virus. The inoculated eggs were candled daily thereafter and the mortality ratios and the average day of death of the various groups of embryos are presented in Table II. It is clear that injec-

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TABLE I. Antiviral Spectrum of Viscosin in Embryonated Eggs as Determined by the Contact Test.

Virus		Route	M. I. C.* (mg)
Miyagawanella felis	1000 LD_{50}	Y. S.	>2
Vaccinia	100	Y. S.	>2
Newcastle disease	100 ID_{50}	A. S.	.125
Influenza A	100	A. S.	< .063
A	1000	A. S.	.25
B	100	A. S.	.063
B	1000	A. S.	.125
Infectious bronchitis	100000 LD_{50}	A. S.	< .063

Y. S. = yolk sac; A. S. = allantoic sac.
* M. I. C. = Minimal inhibiting concentration: smallest amt of viscosin per ml which exerted a definite suppressive effect.

TABLE II. Protective and Therapeutic Effect of Viscosin on Infectious Bronchitis Virus (IBV) in Chicken Embryos.

1st inj.*	2nd inj.*	Time interval, hr	Result D/T	Result A.D.D.
.125 mg viscosin	100 LD_{50} IBV	1	6/26	
.063		1	8/23	
.031		1	25/26	3.2
Saline		1	29/29	2.2
100 LD_{50} IBV	.125 mg viscosin	1	19/22	4.4
	.063	1	24/25	3.7
	.031	1	20/20	2.8
	Saline	1	41/41	2.4
100 LD_{50} IBV	.125 mg viscosin	4	19/21	3.4
	.063	4	27/27	3.5
	.031	4	18/18	3
	Saline	4	40/40	2.7

D/T = No. embryos dead/total. A.D.D. = Avg day of death.
* .5 ml amt via the allantoic sac.

tion of 0.125 or 0.063 mg of viscosin one hour before infection was highly effective in protecting eggs subsequently infected with 100 LD_{50} of virus. However, when viscosin was injected one hour following infection of the embryos its effectiveness was markedly diminished but not abolished. Treatment of the embryos 4 hours after infection was without significant effect. Attempts to demonstrate a protective or therapeutic effect in embryos infected with the influenza viruses or Newcastle disease virus in embryonated eggs were unsuccessful.

Inasmuch as viscosin protected eggs against 100 LD_{50} of infectious bronchitis virus it was of interest to determine whether there had been a reduction in infective titer. Two groups of 10 eggs each received 0.5 ml amounts of phosphate buffer alone and buffer containing 0.125 mg of viscosin, respectively. One hour later both groups of eggs were infected with approximately 10,000 LD_{50} of virus via the allantoic sac. After 24 hours incubation at 36°C(8) the allantoic fluids from 6 viable eggs of each group, respectively, were collected and pooled. On the same day serial decimal dilutions of each pool were inoculated into groups of 7 eggs each via the allantoic sac, and the eggs were candled daily for 6 days. The infective titer of the pooled allantoic fluids from the control group of eggs was $10^{-7.5}$ whereas the titer of the pooled fluids from the treated embryos was less than $10^{-2.0}$. Thus, it is clear that injection of viscosin into the allantoic sac one hour before infection with infectious bronchitis virus greatly reduced the infective titer of allantoic fluid collected from the treated embryos 24 hours after infection.

Infectious bronchitis virus has been shown

8. Groupe, V., *J. Bact.*, 1949, v58, 23.

TABLE III. Effect of Auto-interference on Suppressive Effect of Viscosin on Infectious Bronchitis Virus (IBV).

1st inj. (.5 ml) 1 part Active IBV	9 parts Heated IBV†	2nd inj. (.5 ml) viscosin (mg): .125	.063	.031	.016	Saline
LD_{50} 1000	Undiluted	2/15*	3/13	3/15	1/13	2/13
"	1/3	5/14	3/15	10/14	9/14	8/13
"	1/9	*7/13*	11/14	12/13	14/14	13/14
			D 4.9	D 3.5	D 2.8	D 3.1
"	1/27	*8/15*	12/15	13/13	13/13	11/11
			D 5.1	D 3.3	D 2.8	D 2.3
"	Saline	13/14	9/13	14/14	15/15	25/26
		D 4.6	D 4.6	D 2.9	D 2.6	D 3

* No. eggs dead/total.

† Undiluted allantoic fluid collected from infected eggs held 24 hr at 36°C after death of the embryo and subsequently heated 75 min at 56°C.

D 3: Avg day of embryo deaths = 3 days after inoculation.

to exhibit the phenomenon of auto-interference when allantoic fluid was collected from infected eggs which were held at 36°C for 24 hours after death of the embryo(8). The influence of the interfering material on the antiviral activity of viscosin is shown in the experiment of Table III. When eggs were infected with active virus suspended in subinhibitory concentrations of heated virus and then injected with inhibitory concentrations of viscosin, the total inhibitory effect clearly exceeded that of viscosin alone. Thus, the therapeutic effect of viscosin on infectious bronchitis virus in embryonated eggs was enhanced when subinhibitory concentrations of interfering material (heated virus) were inoculated together with active virus one hour before treatment.

It was of obvious interest to determine the antiviral activity of viscosin *in vivo* in an adult host. In acute toxicity tests the maximum tolerated dose (LD_0) of viscosin for mice was found to be approximately 50 mg per kg when injected intraperitoneally and greater than 250 mg per kg following subcutaneous injection. Necrosis followed by induration was observed at the site of injection in all mice injected subcutaneously with 50 mg per kg or more of viscosin. Oral toxicity was not determined. A preliminary test on the effect of viscosin on the rate of death of groups of 18 mice each infected intranasally with 100 LD_{50} of influenza A virus showed that daily administration of 1 mg subcutaneously resulted in an average day of death of 5.5 days after infection as compared with 4.5 days in the untreated group. A similar delay in the average day of death of 1.0 day was obtained in another experiment. Since the toxicity of viscosin for infected mice could easily increase with the severity of the disease, the time of appearance and the degree of pulmonary consolidation were utilized as criteria for the measurement of possible antiviral activity in the following experiment. Three groups of 36 mice each were infected with approximately 10,000 ID_{50} of influenza A virus intranasally. One group received no further treatment. Mice in the second group received a single injection of 5 mg of viscosin subcutaneously immediately after infection. Mice in the third group received daily subcutaneous injections of 5 mg of viscosin beginning on the day of infection. Twelve mice from each group were sacrificed on the second, third, and fourth day after infection, respectively, as indicated in Table IV. The lungs of each mouse were carefully examined, and the degree of pulmonary consolidation was noted. The data show that daily administration of 5 mg of viscosin resulted in a slight but detectable reduction in the degree of pulmonary consolidation in mice sacrificed on the 3rd but not on the 4th day after infection. When only one injection of the antibiotic was given, the degree of pulmonary consolidation in the treated mice did not differ appreciably from the controls. Similar results were obtained in each of 6 additional experiments.

Discussion. The antibiotic activity of vis-

TABLE IV. Suppressive Effect of Viscosin on Mice Infected with 10000 ID_{50} of Influenza A Virus.

Viscosin	Mice sacrificed after ... days	D/T	I/T	Avg score*
0	2	1/12	4/12	.6
5 mg SC × 1	2	0/11	4/11	.4
2	2	0/12	1/12	.1
0	3	4/12	12/12	2.3
5 mg SC × 1	3	3/12	11/12	2
3	3	0/12	8/12	.7
0	4	11/12	12/12	3.9
5 mg SC × 1	4	8/12	12/12	3.3
4	4	8/12	12/12	3.2

SC = subcut. × 1 = 1 inj. immediately after infection. × 3 = 3 daily inj. beginning on day of infection. D/T = No. of mice dead/total. I/T = No. of mice infected/total.

* Score: 1 = 5-25% lung tissue consolidated; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

cosin was found to be strikingly specific, not only among the bacteria(1,4) but also among the viruses. The most outstanding characteristic of viscosin observed was its marked protective effect in eggs subsequently infected with infectious bronchitis virus. It is of interest in this connection that subinhibitory amounts of interfering material (heated virus) in the viral inoculum enhanced the suppressive effect of inhibitory amounts of viscosin injected one hour after infection in embryonated eggs. The suppressive effect of viscosin on the progress of infection with influenza A virus in mice was detectable but minimal, and interpretation of the results obtained is difficult. While repeated demonstration of a slight suppressive effect cannot be disregarded, little can be learned from the data save that the effect is minimal. Taken as a whole, the data obtained indicate that viscosin shows little promise of any immediate practical application.

Summary. Viscosin was found to exert a marked protective effect in embryonated eggs subsequently infected with infectious bronchitis virus. A slight but detectable suppressive effect on the progress of infection in mice infected with influenza A virus was demonstrated.

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Adrenal Medullary Stimulating Action of Tetraethylammonium.* (19072)

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The primary action of the tetraethylammonium (TEA) ion is ganglionic blockade. This action results in transitory decreases in blood pressure in most species when the intravenous doses are less than 10 mg/kg(1). Administration to animals of larger amounts of the drug, however, frequently produces rises of blood pressures, although the quantity required to elicit a pressor response appears rather variable(1). In addition, pressor responses have been reported in some human subjects given therapeutic amounts(2), and can seemingly be obtained routinely in patients with pheochromocytoma(3). This hypertensive action of TEA has never been adequately explained. It has been attributed to a direct peripheral constricting effect(4), and more recently, to the release of nor-epinephrine primarily from the liver and to a lesser extent from the adrenals(5). No attempt has been made to explain the pressor action on the basis of ganglionic and/or

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