THE ACTION OF TERRAMYCIN ON THE GROWTH OF STRAINS OF INFLUENZA, HERPES SIMPLEX, AND RABIES VIRUSES IN CHICK EMBRYOS AND MICE*

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The value of terramycin as an antibacterial agent has been shown in previous publications[1–3] and in other reports presented in this monograph. It is the purpose of this paper to describe some experiences with terramycin salts and how they influenced the course of a few experimental virus infections. The bulk of our attention has been focused on the action of this drug in influenza virus infections. Mention will also be made, however, of some work done on two strains of herpes simplex virus and a strain of rabies virus.

Material and Methods. The sodium terramycin salt was highly soluble in phosphate saline buffer (pH 7.4). Consequently, this diluent was used in all chick embryo experiments. The final pH of the solutions used was between 8.3 and 8.6. The material inoculated subcutaneously in mice was dissolved in distilled water and had approximately the same pH. All solutions were made up fresh for each inoculation. The viruses used and their passage history are as follows: PR8 strain of type A influenza—Ferrets 198, Mouse 285, Egg 64, for egg inoculation and F 198, M 846 for mouse inoculation; Lee strain of Type B influenza virus, M 137E141; Armstrong strain of herpes simplex virus, M 49E31, M5E6; HF strain of herpes simplex virus, M?M5; rabies virus, passed in mice with passage once a month in rabbits, number of passages not known.

Experiments with Chick Embryos. The volume of drug solution injected in all instances was 0.2 ml. and contained the desired dosage of terramycin. The interval between injection of terramycin and virus was varied and is discussed under results. Most injections of both virus and terramycin were made into the allantoic cavity of 10 to 12 day-old embryos. A few tests utilizing the yolk sac as the route of injection were done and resulted in more deaths than by the allantoic method. In all tests, simultaneous controls of drug alone and virus alone were also carried out. Furthermore, preliminary tests for the toxic effect of drug alone were carried out in groups of 8 to 20 eggs.

Influenza virus was diluted 10-fold in nutrient broth, and herpes simplex in 10 per cent horse serum in saline. The volume of inoculum was 0.2 ml., and 4 to 6 eggs were used for each dilution of virus. The eggs were incubated for 40 hours at 35°C. for influenza and 72 hours at 37°C. for herpes. The eggs were chilled for 2 hours at 4°C. in those instances where allantoic fluids were to be removed. Such fluids were tested by diluting 1 to 4 with

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saline and 0.5 per cent chicken erythrocytes. The endpoints were calculated by taking the 50 per cent infectious dose,[4] arrived at by the number of fluids exhibiting clear evidence of hemagglutination. The endpoint for the herpes-infected eggs was taken as those eggs showing definite evidence of the growth of multiple pocks on the chorio-allantoic membrane.

Experiments with Mice. Swiss albino mice, weighing from 14 to 20 grams, were infected by either the intranasal inoculation of 0.05 ml. of virus, diluted in 10 per cent horse serum saline, or the intracerebral inoculation of 0.03 ml. of virus suspension. Terramycin was administered in 0.5 ml. amounts subcutaneously at either 8- or 12-hour intervals and given for 4 to 6 days after the animals were infected. Groups of 10 to 12 mice were used for each 10-fold dilution of virus, and the 50 per cent lethal endpoint was calculated after observation of the mice for 6 days, in the case of rabies-infected animals, and 10 days for the influenza- or herpes-infected animals. The animals that did not receive injections of drug were given, subcutaneously, distilled water in a schedule corresponding to that of the drug-treated animals.

Results with Influenza Virus. The effect of sodium terramycin on 10 to 12 day-old chick embryo showed that, when injected into the allantoic sac of 8 eggs, in 20 mg. amounts, 3 of the 8 were dead after 40 hours' incubation. At 15 mg. doses, 3 of 8 were moribund, and, in 10 and 5 mg. amounts, occasional eggs were moribund. Despite this obvious toxicity, 10 day-old embryos, in groups of 6, were given allantoic injections of 10, 15, and 20 mg. amounts of sodium terramycin and, one hour later, 10 ID_{50} of the PR8 strain of Type A influenza virus. When the allantoic fluids were removed after 40 hours' incubation and tested for hemagglutination, they showed that, with this small inoculum of virus, there was no evidence of multiplication in the presence of any of the three concentrations of drug. When the eggs were harvested, it was noted that a dark brown precipitate adhered to the chorio-allantoic membrane. It was assumed that this represented some of the terramycin that had been injected and partially precipitated and possibly represented incomplete utilization of the drug. Eggs injected *via* the yolk sac showed the same precipitate. A test using 1000 ID_{50} of the same virus given one hour after the drug showed suppression of growth only in eggs that had received 20 mg. of terramycin.

The influence of this drug on the respiration of chorio-allantoic membrane, as measured in the Warburg apparatus, has shown no inhibitory effect on O_2 uptake or glucose utilization for an interval of six hours when as much as 1.0 mg. of terramycin per 200 mg. of membrane has been used.* When virus is added to flasks containing pieces of chorio-allantoic membrane in the proper buffering system, consistent multiplication of approximately 5 to 6 logs occurs. When terramycin in concentrations of 0.3 mg./flask is added, however, there is no evidence of virus multiplication (as measured by hemagglutination test) although the original inoculum of virus can be recovered, indicating that the drug exerts no influence on the virus itself but acts by interfering between virus and tissue cells.

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The results of other experiments, where groups of 10 day-old embryos were injected with 5, 10, and 15 mg. amounts of drug and 1 hour later given decreasing 10-fold concentrations of virus, are shown in TABLE 1. The eggs that received 5 mg. of terramycin showed a virus titration 10-fold lower than the untreated eggs, and the 10 and 15 mg. groups had titers that were 1000-fold below the controls, clearly indicating suppression of virus growth. The same concentrations of drug alone resulted in death of some of the embryos, emphasizing the fact that the levels of terramycin used were definitely toxic. The influence of time of injection of the drug is also important, for, when the virus was given 3 hours before the terramycin, there was no significant difference between the final titer of treated and untreated eggs, regardless of the amount of drug added. The implication of these findings makes it appear likely that this effect of terramycin on the

TABLE 1

EFFECT OF SODIUM TERRAMYCIN ON THE GROWTH OF THE PR8 STRAIN OF TYPE A INFLUENZA VIRUS IN CHICK EMBRYOS

Amt. of drug	*Drug controls*		*Time of inoc. of drug into allantoic sac*	*Final ID_{50} virus titer*
Control	*Moribund*	*Dead*		10^{-8}
5 mg./egg	0/20	0/20	1 hr. before virus	10^{-7}
10 mg./egg	4/20	1/20	1 hr. before virus	10^{-5}
15 mg./egg	7/20	4/20	1 hr. before virus	10^{-5}

Amt. of drug	*Drug controls*		*Time of inoc. of drug into allantoic sac*	*Final ID_{50} virus titer*
Control	*Moribund*	*Dead*		$10^{-7.5}$
5 mg./egg	1/20	0/20	3 hrs. after virus	$10^{-7.0}$
10 mg./egg	3/20	0/20	3 hrs. after virus	$10^{-6.7}$
15 mg./egg	5/19	2/19	3 hrs. after virus	$10^{-7.0}$

growth of influenza virus depends in large part on whether virus or host cells can be acted upon before an opportunity occurs for the virus to initiate one growth cycle. In this respect, a test with the Lee strain of Type B influenza virus, where the first growth cycle has been shown to be slower than with PR8,[5] showed a slightly greater suppression of virus multiplication when 10 or 15 mg. of terramycin was injected 3 hours after the virus.

Despite the interesting effects of terramycin on the growth of influenza virus in chick embryos, the results of studies done in mice are much less impressive. TABLE 2 shows the effect of drug given every 8 hours subcutaneously. There is obviously no significant difference in the titers of the treated and untreated animals. However, the concentrations of terramycin were quite low. In TABLE 3, the effect of larger amounts of terramycin also shows no significant influence, despite the fact that such dosages are approaching the acute toxic concentration of drug for these animals.

Results with Other Viruses. TABLE 4 gives the effect of terramycin on

the growth of the Armstrong strain of herpes simplex virus and shows no difference in the final titer between treated and untreated groups of eggs. The terramycin was injected into the allantoic sac 1 hr. before the virus was inoculated onto the chorio-allantoic membrane.

The HF strain of herpes simplex virus injected intracerebrally in mice resulted in the same titer in animals that received 0.5 mg. of terramycin subcutaneously every 12 hours for 6 days as in the animals that received

TABLE 2

EFFECT OF SODIUM TERRAMYCIN ON THE GROWTH OF THE PR8 STRAIN OF INFLUENZA VIRUS IN WHITE MICE

Amt. of drug	*Drug controls dead*	*Number of subcut. injections every 8 hrs.*	*Final ID_{50}* virus titer*
Control			$10^{-6.2}$
.1 mg./mouse	0/20	15	$10^{-6.4}$
.25 mg./mouse	0/20	15	$10^{-6.6}$
.50 mg./mouse	0/20	15	$10^{-6.6}$

* Virus given intranasally in 10-fold dilutions to groups of 10 mice after the 4th dose of drug.

TABLE 3

EFFECT OF LARGER DOSES OF SUBCUTANEOUS SODIUM TERRAMYCIN GIVEN EVERY 12 HOURS ON THE GROWTH OF INFLUENZA VIRUS IN MICE

Amt. of drug	*Drug controls*	*No. injctions*	*Final LD_{50} virus titer*
Control			$10^{-7.4}$
.75 mg./mouse	0/10	12	$10^{-7.1}$
1.00 mg./mouse	0/10	12	$10^{-7.8}$
1.50 mg./mouse	0/10	12	$10^{-7.4}$

TABLE 4

EFFECT OF SODIUM TERRAMYCIN IN ALLANTOIC CAVITY ONE HOUR BEFORE ARMSTRONG STRAIN OF HERPES SIMPLEX

Amt. of drug	*Time of inoc. of drug into allantoic sac*	*Final titer of virus*
Control		$10^{-5.2}$
10 mg.	1 hour before virus	$10^{-5.0}$
15 mg.	1 hour before virus	$10^{-5.2}$

no drug. However, the treated animals that received the more dilute virus tended to live longer than the corresponding animals in the control group.

When mice were inoculated intracerebrally with 0.03 ml. of 10-fold dilutions of rabies virus, the control animals developed an LD_{50} of $10^{-2.6}$. Terramycin given in 1.5 mg. amounts subcutaneously 1 hour before virus and every 12 hours thereafter for 6 days did not influence the final titer of virus.

Summary

The effect of the sodium salt of terramycin on experimental infection of chick embryos with influenza virus results in suppression of viral multiplication if the toxic level of the drug is approached and if the material is given before the virus. No effect was observed on the course of Type A influenza virus infection in mice; on the growth of strains of herpes simplex virus in chick embryos and mice; or on the multiplication of a strain of rabies virus in mice.

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