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Ineffectiveness of Aureomycin and Terramycin Against Rabies Street Virus in Mice.* (19824)

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There is no therapeutic material that is efficacious against infection with rabies virus. Therefore it was of interest to try terramycin for two reasons: a) concentration of ingested terramycin in the brain is reported to be relatively high(1), and b) conflicting evidence has been presented on the efficacy of terramycin against rabies virus. Quilligan *et al.*(2) showed no effect of terramycin on the titration of rabies virus in mice, while Azevedo and de Macedo(3) claimed 98% survival rates in rats and rabbits. This work was undertaken to secure more evidence on the action of terramycin on the rabies virus, and to compare it with aureomycin.

Method and materials. The virus material was rabies street virus in a 20% suspension of dog salivary gland.‡ The LD_{50} of this virus by intracerebral inoculation in 3-week-old white mice was $10^{-5.8}$ as calculated by the Reed-Muench method(4). The terramycin and aureomycin were incorporated into finely ground fox-chow at the rate of 100 mg of antibiotic per 100 g of feed. The mice were starved for a day prior to the experiment so that they would accept the ground mixture. In order to get some idea of how much drug the mice were ingesting, a weighed amount of feed and material was placed in a Petri dish and the material was weighed each day. The difference was then taken to be the amount consumed by the mice in the cage. The amount of aureomycin or of terramycin actually ingested by mice under this regimen is adequate to ensure a definite therapeutic response with murine and scrub typhus infections(5).

Procedure. Twenty mice divided into 2 cages with 10 mice in a cage were placed on the aureomycin feed mixture. Twenty mice divided into 2 cages with 10 mice in a cage were placed on the terramycin feed mixture. Twenty mice divided into 2 cages with 10

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TABLE I.

		Avg day of death	Stand. dev.
Terramycin	Cage I	12.3	1.9
	" II	12	1.8
Aureomycin	" I	12.1	2.2
	" II	11.9	1.5
Controls	" I	12.1	1.7
	" II	11.8	2.4

mice in a cage were used as controls. All 60 mice were inoculated in each masseter muscle with 0.05 ml of a 10^{-3} dog salivary gland suspension whose LD_{50} was measured by intracerebral titration was $10^{-5.8}$. Therefore each mouse received 0.1 ml of the salivary gland suspension or about 1200 intracerebral LD_{50} doses. The salivary gland was diluted with 10% inactivated rabbit serum in buffered saline solution (rabbit serum heated at 50°C for 15 minutes; the saline was buffered at pH 7.4). A second experiment was set up to determine the effect of contact of terramycin on rabies virus. The salivary gland suspension was diluted with sterile distilled water suspension of terramycin so that each cubic centimeter of final salivary gland suspension had 300 gamma of terramycin per ml. The final pH of this solution was 3.8. Control salivary gland suspensions without terramycin were made up to pH 3.8. Both the terramycin-salivary gland suspension and the control suspensions were incubated at 37°C for 2 hours. The final salivary gland concentration in both suspensions was 10^{-3}. Twenty mice were inoculated with the terramycin-salivary gland suspension at the rate of 0.05 ml into each masseter muscle. The 20 mice were divided into 2 cages of 10 each. The salivary gland suspension at pH 3.8 without terramycin was given to 20 other mice and these two were divided into two cages of 10 each. The observation period was 30 days. Each mouse that died was autopsied and the brain examined by means of impressions stained with Seller's stain and searched for Negri bodies.

Results. All Table I mice in the therapeutic trials died. Table shows average day of death in each category, 10 mice in each experiment.

All mice when autopsied showed Negri bodies when impression smears of the brain were stained with Seller's stain and examined microscopically.

The quantity of antibiotics ingested was roughly in the order of 200 γ per mouse per day. On the average the mice weighed approximately 15 g each and were predominantly males.

In the experiment where the terramycin was mixed with the virus suspension 4 mice died within the observation period of 30 days. Two of the controls died. Impression smears made of the brains of these animals showed Negri bodies when stained with Seller's stain. The survivors which were sacrificed at 31 days showed no Negri bodies.

Discussion and summary. 1. Neither aureomycin nor terramycin administered as described had an effect on the course of rabies in white mice when street virus was inoculated into the masseter muscle. 2. The pH of the terramycin preparation tried was sufficiently low to exert a lethal action on the street virus. A control suspension buffered at the same pH as the terramycin solution exerted the same lethal effect on the street virus.

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