15805

Streptomycin in Treatment of Experimental Trypanosomiasis in White Mice and Chick Embryos.*

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The striking results obtained by the use of streptomycin and penicillin, in certain bacterial diseases, has somewhat overshadowed their use against fungus and protozoan infections. With respect to trypanosomiasis, Neghme,[1] working with *T. cruzi* in mice, and Augustine, *et al.*,[2] investigating *T. lewisi* in rats, have reported completely negative results in the treatment of experimental trypanosomiasis with penicillin. More recently Schatz and colleagues[3] have described an attempt to isolate an antibiotic active against *T. equiperidum*. In their work they found that a crude preparation of streptomycin immobilized the trypanosomes in freshly drawn rat blood but only when used in a concentration greater than 1:200. Anderson, Villela and Reed[4] were unable to show cytolysis of *T. equiperidum* by penicillin or streptomycin; and subtilin, in their hands, failed to prolong the life of mice infected with this organism. The use of streptomycin in human trypanosomiasis, particularly Chagas disease, has been somewhat limited but from information available,[5] the results have been regularly disappointing.

The following report presents the results of a study on the influence of streptomycin in experimental infections of mice and chick embryos with *T. brucei, T. equiperidum* and *T. hippicum*.† These flagellates are maintained in the laboratory stock culture collection by serial passage in guinea pigs. In the present study the blood of infected rats was used as the source of organisms.

Experimental Procedure. White Mice. White mice were inoculated subcutaneously with blood taken from the tail of an infected rat. Streptomycin was administered subcutaneously at 3-hour intervals, for a period of 4 days, beginning 24-36 hours after the preliminary inoculation. Total dosage ranged up to 16,000 units per mouse. Addinall[6] gives the LD_{50}‡ for mice as 750 mg per kg when given subcutaneously. Two control series were included in each experiment; (1) the trend of the infection in untreated mice and (2) the toxicity of the antibiotic for normal mice. The course of the disease was followed by making microscopical examinations of blood specimens taken, by snipping the tip of the tail, at 12- to 24-hour intervals. These examinations were made with the aid of dark-field illumination and the number of trypanosomes per oil immersion field (diameter 145 μ) was determined and recorded.

Chick Embyros. Fertile eggs, which had been incubated 10 days, were inoculated with heart's blood from an infected rat. The material was injected directly into the yolk sac using a 1½-inch, 20-gauge needle and entering from the air sac end of the egg. This

* Streptomycin, as the hydrochloride, was made available for this study through the courtesy of Merck and Co. and Abbott Laboratories.

1 Neghme, Amador, *Science*, 1945, **101**, 115.

2 Augustine, D. L., Weinman, D., and McAllister, J., *Science*, 1944, **99**, 19.

3 Schatz, A., Magnuson, H. J., Waksman, S. A., and Eagle, H., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 143.

4 Anderson, H. H., Villela, G. G., and Reed, R. K., *Science*, 1946, **103**, 419.

5 Personal communication of Dr. M. H. Soule.

† The original sources of the trypanosome strains used in this study were: (1) *T. brucei*—lineally descended from the original isolation of Bruce (1896). Obtained from Dr. Thomas, Liverpool School of Tropical Medicine, about 1900. (2) *T. equiperidum*—from Dr. A. L. Tatum, University of Wisconsin. (3) *T. hippicum*—from Dr. Herbert Clark, Gorgas Memorial Institute, Ancon, Canal Zone.

6 Addinall, C. R., *The Merck Report*, October, 1945.

‡ Lethal dose for 50% of mice treated.

TABLE I.
The Effect of Stroptomycin on Various Trypanosome Infections in White Mice.

Days after inoculation	2	3	4	5	6	7	8	9
T. brucei + streptomycin	0/s*	1/f†	15/f	AC‡	\§	Dead		
" " control	0/s	1/s	1/5f	10/f	\	"		
T. equiperidum + streptomycin	1/s	1/10f	3/f	AC	AC	"		
" " control	1/s	1/10f	2/f	15/f	AC	"		
T. hippicum + streptomycin	0/s	0/s	1/s	\	1/10f	10/f	25/f	Dead
" " control	0/s	0/s	1/s	\	10/f	AC	AC	"

* 0/s = no trypanosomes present on slide.
† 1/f = 1 trypanosome per oil immersion field.
‡ AC = too numerous to count.
§ \ = no count made.

TABLE II.
Effect of Streptomycin on Various Trypanosome Infections in Chick Embryos.*

Days after inoculation	3	4	5	6	7	8	9
T. brucei + streptomycin	0/s	0/s	\	1/10f	5/f	Dead	
" " control	0/s	0/s	\	2/f	5/f	"	
T. equiperidum + streptomycin	0/s	1/s	2/f	3/f	15/f	\	Dead
" " control	0/s	1/10f	5/f	10/f	15/f	\	"
T. hippicum + streptomycin	0/s	\	0/s	\	1/s	1/f	"
" " control	0/s	\	0/s	\	1/10f	1/3f	"

* See footnotes to Table I.

hole was immediately sealed with sterile paraffin. The streptomycin was introduced through the same opening as the original inoculation after thorough application of tincture of iodine to the paraffin seal. Three treatments were given every 24 hours for a period of 4 days. The hole was resealed after each injection of streptomycin. The total dosage in several instances was 40,000 units. The same controls, *i.e.* normal infection and toxicity, were used as were included in the mouse series.

Samples of blood, for microscopic examination, were obtained by nicking the shell of the egg with a carborundum disc previously treated with tincture of iodine.[7] The openings were made in a region rich in capillaries but not overlying the large vessels. The orifice was, in each case, carefully sealed with sterile paraffin. Uninfected embryos were subjected to the same treatment, sterile saline solution being substituted for the streptomycin. These embryos served to indicate damage due to trauma. The microscopic studies were made with the aid of dark-field illumination and the results recorded as previously described.

Results. Typical examples of the findings, with the infection in mice, are presented in Table I. Each of the animals received 600 units of streptomycin at 8:30 a. m., 11:30 a. m., 2:30 p. m. and 5:30 p. m. and 1,600 units at 8:30 p. m. for a period of 4 days, making a total of 16,000 units for each mouse.

As shown by Table I, there was no significant difference in either the course or the duration of the trypanosome infection in treated and untreated mice. In certain other experiments, using *T. brucei,* a total quantity of 28,000 units of streptomycin per mouse was administered with the same results. The toxicity controls showed no ill effects from any of the quantities used.

Table II gives a typical example of the results of the experiments with chick embryos. Each embryo was treated with 2,000 units of streptomycin at 10:30 a. m. and 2:30 p. m. and 6,000 units at 5:30 p. m. for a period of 4 days, making a total of 40,000 units for each embryo.

The data in Table II clearly indicate that there was no significant difference in the course or duration of the infection between treated and untreated embryos. The toxicity controls survived and many hatched.

The data recorded in this series of experiments confirm the findings of Hood,[7] who

[7] Hood, Mary N., unpublished thesis.

made a thorough investigation of the course of infection of trypanosomiasis in animals and chick embryos with the same strains of organisms used in this study.

Summary. White mice and chick embryos were infected with *T. brucei, T. equiperidum* and *T. hippicum.* After a lapse of 24-36 hours streptomycin was administered subcutaneously to the mice and was injected into the yolk sac of the chick embryos. Each mouse received a total of 16,000 units and each chick embryo, a total of 40,000 units of streptomycin. The course of the disease was followed by microscopic examination of blood specimens taken at regular intervals. As far as could be determined, this antibiotic agent did not alter the course of the infections or prolong the life of the treated mice or embryos.

15806

Low Toxicity of Sulfonamide Mixtures. II. Combinations of Sulfathiazole, Sulfadiazine and Sulfamerazine.*

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A new and simple approach to the prevention of renal complications caused by sulfonamides was presented in a previous communication.[1] It consists in the use of *mixtures* of sulfonamides instead of single compounds. The idea emerged from the observation that a saturated aqueous or urinary solution of a sulfonamide could still be fully saturated with a second and third sulfonamide of different molecular structure, each compound behaving as though it were present alone and exerting no influence on the solubilities of the others. Consequently, in solutions containing several sulfonamides, the maximum obtainable concentration appeared expressed by the sum of the solubilities of all the drugs present. This finding applied to the free compounds, as well as to their acetylated homologues.

It was reasoned on the basis of this observation that the danger of intrarenal formation of sulfonamide crystals could be considerably reduced by employing combinations of *partial* dosages of 2 or more therapeutically equivalent sulfonamides instead of single compounds. The validity of this contention was demonstrated in experimental and clinical studies with a mixture of sulfathiazole and sulfadiazine.[2,3]

The present paper deals with the toxicity of a sulfadiazine-sulfamerazine and a sulfathiazole-sulfadiazine-sulfamerazine combination. It contains also some additional data on the sulfathiazole-sulfadiazine mixture, in particular a comparative quantitative estimation of intrarenal sulfonamide deposits from sulfathiazole, sulfadiazine, and their combination.

Materials and Methods. Four hundred albino rats from our own colony, 8-12 weeks old and weighing between 160-210 g were employed in all experiments with the exception of the study on chronic toxicity in which weanling rats were used. The animals were kept on a standard diet (Rockland Farms Rat Diet) and had free access to water.

For determination of the acute toxicity, observation was continued for 5 days following a single intraperitoneal injection of the sulfonamides because of the well known de-

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1 Lehr, D., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 11.

2 Lehr, D., *J. Urol.*, 1946, **55**, 548.

3 Lehr, D., Slobody, L. B., and Greenberg, W. B., *J. Pediat.*, 1946, **29**, 275.