Effect of Erythromycin, Thiocymetin, and Three Other Antibiotics on *Leptospira icterohemorrhagiae* in the Chick Embryo. (20501)

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The leptospiroses form a clinical complex which is world-wide in distribution and has been found in every geographical region of the United States. Only recently has it been realized that the disease is not only widespread in the continent but that the incidence is considerably higher than had been generally known both in animal and man(1).

Penicillin and streptomycin have heretofore been considered to be among the more effective drugs in the treatment of the disease(2,3). Aureomycin has been shown to be effective in terminating the carrier condition in experimentally induced *L. canicola* infections in hamsters and dogs(4). Chloramphenicol has been reported to be ineffective in experimental infections with *L. icterohemorrhagiae* in hamsters and guinea pigs and in eradicating the carrier state in dogs(5). With respect to human leptospirosis, the statement has been made that none of these antibiotics has been shown to be particularly promising as an effective drug for the treatment of clinical cases(5).

It therefore became of interest to examine the effect of two new compounds, erythromycin and thiocymetin, on *Leptospira icterohemorrhagiae,* the causative agent of Weil's disease in man, which along with *L. canicola* and *L. pomona* and other species, causes leptospirosis in both animals and man. This communication reports the relative potencies of erythromycin(6,7), thiocymetin(8,9), chloramphenicol, aureomycin, streptomycin, and penicillin G in terms of their ability to delay the death of chick embryos infected with *L. icterohemorrhagiae.*

Materials and methods. The compounds tested were erythromycin, Dd-threo-2-dichloroacetamido-1-(4-methylsulfonylphenyl)-1, 3-propanediol or thiocymetin, chloramphenicol, aureomycin HCl, the potassium salt of penicillin G, and streptomycin trihydrosulfate.

A serologically typical strain (L204) of *L. icterohemorrhagiae* which was originally isolated from a rat and which is virulent in guinea pigs was employed in all tests.

The tests were carried out by injecting groups of 25 to 30 6-day-old chick embryos via the yolk sac with 0.2 ml aliquots of a suspension of *L. icterohemorrhagiae* of such a concentration that the mean death time in control groups averaged 4.7 to 4.8 days. Twenty-four hours after injection of the organisms, the embryos were similarly injected with aqueous solutions of drug given in 0.5 ml aliquots and in amounts not exceeding the previously determined maximum tolerated dose. All embryos dying within 72 hours after injection of the organisms were discarded and not counted in the test results on the basis that such deaths (which averaged 15%) were due to trauma of injection. The embryos were then candled daily for 13 days, at which time tests were terminated. Embryos still alive on the 13th day were considered dead on the 14th day for purposes of calculation. The difference between the mean death time of the control groups and the treated groups was taken as a measure of the therapeutic effectiveness of the drug.

Experimental results. A summary of the test results is presented in Table I. The standard error of the mean (σ_m) in the control groups ranged from $\pm$ 0.05 to $\pm$ 0.09 days. In the experimental groups σ_m was somewhat larger due to increased scatter of the data. In the tests with all compounds except erythromycin, σ_m was $\leqq \pm$ 0.4 days. In the erythromycin tests, σ_m was $\leqq \pm$ 0.6 days.

As can be seen in Table I, the compounds may be ranked in the following order of effectiveness on the basis of equi-molar dosages: erythromycin >penicillin G >streptomycin >aureomycin >thiocymetin >chloramphenicol.

The data in Table I indicate that erythromycin in the chick embryo was of the order of 8 to 40 times more effective on an equi-molar

TABLE I. Effect of Erythromycin, Penicillin G, Streptomycin, Aureomycin, Chloramphenicol, and Thiocymetin on Mean Death Time of Chick Embryos Infected with *L. icterohemorrhagiae*. MD = mean death time in days.

Compound tested	μg/egg	μM/egg	No. of tests	MD, exp.*	MD, control ($\sigma_m \gtrless \pm .09$)	Delay in MD
Erythromycin	11	.015	1	7.7 (0/23)	4.7	3.0
	22	.03	1	9.9 (0/22)	4.7	5.2
	40	.06	1	≷ 12.3 (5/24)	4.8	≷ 7.5
	80	.11	1	≷ 13.1 (25/31)	4.8	≷ 8.3
Penicillin G	90	.24	1	≷ 9.5 (2/24)	4.7	≷ 4.8
	900	2.40	1	≷ 12.6 (18/24)	4.7	≷ 7.9
Streptomycin	220	.25	1	≷ 7.5 (1/25)	4.7	≷ 2.8
	2200	2.50	1	≷ 11.0 (7/25)	4.7	≷ 6.3
Aureomycin	129	.25	1	6.9 (0/23)	4.7	2.2
	250	.49	4	8.9 (0/140)	4.8	4.1
	515	1.00	1	10.7 (0/21)	4.7	6.0
Chloramphenicol	81	.25	1	5.2 (0/24)	4.7	.5
	808	2.50	1	9.2 (0/22)	4.7	4.5
Thiocymetin	100	.28	2	5.7 (0/53)	4.8	.9

* Figures in parentheses refer to number of embryos alive on 13th day post-inoculation over total number of embryos used in test.

basis than penicillin G, and 17 to 40 times more effective than streptomycin or aureomycin.

Streptomycin and aureomycin in these tests were not significantly different in their effect and both were definitely less effective than erythromycin or penicillin G.

Chloramphenicol and thiocymetin were not significantly different from each other in their effect at equi-molar concentrations. The delay in death afforded by these compounds at doses of 0.25-0.28 μM was probably not significant. Chloramphenicol at a dose of 2.5 μM however, gave a significant delay in mean death of 4.5 days. Thiocymetin could not be tested at a dose higher than 0.28 μM since this is the maximum tolerated dose for chick embryos.

Summary and discussion. Five broad spectrum antibiotics and chemotherapeutic agents have been tested for their relative effectiveness in suppressing *L. icterohemorrhagiae* infections in chick embryos. The drugs may be ranked in the following order of potency: erythromycin >penicillin G >streptomycin >aureomycin >thiocymetin >chloramphenicol. These results indicate that erythromycin is potentially a valuable aid to leptospirosis therapy. This must be tested in experimental animals in comparison with penicillin and streptomycin before any more definite statement can be made.

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