

17279. Aureomycin in Experimental Polyarthrititis with Preliminary Trials in Clinical Arthritis.*†

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A polyarthrititis of rats can readily be reproduced by intraperitoneal or intravenous injection of broth cultures of the L₄ strain of pleuropneumonia-like organism. This experimental polyarthrititis has been used as a means of making chemotherapeutic trials.¹⁻³ In man pleuropneumonia-like organisms have repeatedly been isolated from the genitourinary tract and "may be related etiologically to an acute infectious type of arthritis and to Reiter's syndrome."⁴ Dienes has isolated L type cultures from many Gram negative bacilli and several large Gram positive bacilli,⁵ although thus far no one has reported isolation of such forms from streptococci. Unfortunately these microorganisms are species specific as far as their pathogenicity is concerned so that human strains do not produce infections in animals. While the polyarthrititis of rats is not the same disease as rheumatoid arthritis in man, the course of both is favorably altered by the use of gold salts. In the rat the arthritis may be prevented by the intramuscular injection of gold at the time of infection, or after the arthritis is developed, treatment with gold will promote healing more

rapidly than in untreated controls.¹ Our effort in the chemotherapy of this rat arthritis has been directed toward finding agents which might be as effective as gold, but less toxic, for possible trials in rheumatoid arthritis of man. The new antibiotic, aureomycin, has proved to be successful in this experimental polyarthrititis.

Methods. Aureomycin was given to the infected rats by stomach tube, by subcutaneous injection, or by mixing with the diet. The effect of aureomycin both as a preventive and a curative agent was evaluated. Scoring was designed to include the per cent incidence of arthritis in each group of white rats tested, and the per cent survival. The extent of the joint involvement was evaluated according to a modification of the arthrogram of Sabin and Warren⁶ which assigns a numerical value of 4 to each front leg and 5 to each hind leg, giving a total of 18 points per animal assuming maximal joint involvement of all joints. The average arthrogram scores were determined by adding the total scores for each group and dividing by the number of animals in the group irrespective of per cent incidence of arthritis.

The microbe was cultured according to a method described by Tripi and Kuzell¹ in a broth culture which is a buffered yeast-extract tryptose base enriched with 20% filtered horse serum. Table I shows the protective effect of aureomycin given coincident with the intraperitoneal injection of 2 cc of a broth culture of the microorganism or shortly thereafter.

Results. When 0.1% or 0.3% aureomycin was mixed with the ground Purina dog chow complete protection of all animals occurred (Table I). When aureomycin was adminis-

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† The aureomycin was supplied by Lederle Laboratories Division of the American Cyanamid Company, Pearl River, N. Y.

¹ Tripi, H. B., and Kuzell, W. C., *Stanford Med. Bull.*, 1947, **5**, 98.

² Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., *Brit. J. Exp. Path.*, 1940, **21**, 13.

³ Sabin, A. B., and Warren, J., *Science*, 1940, **92**, 535.

⁴ Dienes, L., Ropes, M. W., Smith, W. E., Madoff, S., and Bauer, W., *New England J. Med.*, 1948, **238**, 509 and 563.

⁵ Dienes, L., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71**, 30.

⁶ Tripi, H. B., Gardner, G. M., and Kuzell, W. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, **70**, 45.

TABLE I.
Preventive Effect of Aureomycin in Polyarthritis of Rats.

% aureomycin in diet	No. of animals*	Procedure	Incidence of arthritis, %	Avg arthrogram score	Survival, %
0.3	20	Aureomycin added on day of infection and continued for 7 days	0	0	100
0.1	20	Aureomycin added on day following infection and continued for 11 days	0	0	100
0.05	26	Aureomycin added on day of infection and continued for 14 days	15.0	0.07	100
0	10	Intubation } Aureomycin given dose } by stomach tube 50 mg/kg } (single daily dose) for 2 days fol- 0 } lowing infection	20.0	0.4	100
0	10	20 mg/kg }	20.0	0.38	100
0	10	Single dose of aureomycin (100 mg/kg) subcutaneously on day of infection	20.0	0.30	100
0	76	Untreated controls	77.4	2.41	96

* The average weight of the groups at the beginning was 67 to 75 g.

tered by stomach tube to fasting animals in single daily doses of 50 mg per kg for 2 days, there was an incidence of 20% arthritis with an average arthrogram score of 0.4, while the incidence among the controls was 77.4%, and the average arthrogram score was 2.41, the survival rate being 96%. A single dose of aureomycin, 100 mg per kg, given subcutaneously on the day of infection gave an incidence of 20% and an average arthrogram score of 0.3, compared to a much higher value (2.41) for the controls (Table I).

The curative effect was evaluated by giving aureomycin 100 mg per kg subcutaneously on the 7th and 8th days after the infection was begun and at a time when there was a 55% incidence of arthritis. The crude aureomycin being quite acid caused local necrosis, so further injections were not given. In 4 days the incidence of arthritis had fallen from 55 to 5% in the treated group while it had increased from 66 to 73% in the controls (Table II). At the same time the average arthrogram score for the treated animals had decreased in 4 days from 1.6 to 0.15 while in the controls it had increased from 1.3 to 1.35. At the outset there was a less severe degree of arthritis in

the controls, the drug being tested against a more severe arthritis, yet the outcome was more favorable.

In vitro, a concentration of 2 μ g aureomycin per cc of the broth permitted questionable growth of the microbes in 24 hours and definite growth in 48 hours as estimated by the turbidity of serial dilutions. Using 3 μ g of aureomycin per cc of broth, no growth was observed.

Estimations of blood levels[‡] of aureomycin in the treated rats, according to the method of Brainerd *et al.*,⁷ showed that rats receiving 0.1% aureomycin in the Purina dog chow for 3 days had blood levels of 0.62 μ g per cc, while those eating a diet containing 0.075% aureomycin had less than 0.155 μ g per cc. Rats receiving 50 mg per kg with a stomach tube once daily for 2 days showed blood levels of 0.62 μ g of aureomycin per cc when the sample was taken 2 hours after the adminis-

‡ We are indebted to Miss Mirra Scaparone of the San Francisco Hospital for determination of the aureomycin blood levels.

⁷ Brainerd, H. D., Bruyn, H. B., Jr., Meiklejohn, G., and Scaparone, M., *Proc. Soc. Exp. Biol. and Med.*, 1949, **70**, 318.

TABLE II.
Curative Effect of Aureomycin in Polyarthrititis of Rats.

	No. of animals*	Incidence of arthritis		Avg arthrogram score	
		Pretreatment, %	4 days after aureomycin, %	Pretreatment	4 days after aureomycin
100 mg aureomycin per kg subcutaneously on 7th and 8th days after infection	20	55	5	1.6	0.15
Untreated controls	20	66	73	1.3	1.35

* The average body weight in both groups was 75 g.

tration of the drug. The untreated controls showed no measurable level of the antibiotic.

Preliminary clinical trials were made in 4 advanced cases of rheumatoid arthritis which had responded unsatisfactorily to several therapeutic agents, and one early case using 2 g aureomycin daily by mouth for 1 month. Two patients noted an improvement in appetite, and one patient had to discontinue the medication on the third day due to a marked gastrointestinal upset. None of these cases showed any improvement in range of motion of affected joints and 3 of them developed increased pain and swelling while under treatment. One case of Reiter's syndrome responded dramatically, gaining 6 lb during the first week of treatment and losing all joint pain. At the end of one month he showed no

more joint swelling. His blood sedimentation rate (Wintrobe Method) decreased from 40 mm to 12 mm per hour in 2 weeks.

Summary. 1. The addition of aureomycin to the diet, subcutaneous administration, and gastric intubation of the antibiotic prevented and cured experimental polyarthrititis of rats due to the L₄ strain of pleuropneumonia-like organism.

2. *In vitro* aureomycin prevented growth of the microbe in broth.

3. In preliminary clinical trials, several patients with chronic rheumatoid arthritis who responded unsatisfactorily to several therapeutic agents also failed to respond to aureomycin.

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17280. Effect of Incubation on the Cholesterol Partition in Human Serum.*

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Sperry¹ reported that the incubation of blood serum or plasma from normal human subjects resulted in a decrease in the amount of free cholesterol present without a change in the total cholesterol. It was concluded that esterification of some of the free chole-

sterol had taken place and that this had come about through the action of an enzyme, as the process was abolished by heating the serum to 55-60° prior to incubation. If this esterification of cholesterol *in vitro* were the result of enzymatic activity, it seemed possible that the enzyme might be absent or present in reduced amounts in the peripheral blood of patients with an abnormally low cholesterol ester fraction in the serum. This would

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¹ Sperry, W. M., *J. Biol. Chem.*, 1935, **111**, 467.