Summary. The 3 steroid compounds, Pregnenetriolone Acetate, 17-Alpha Hydroxyprogesterone, and Compound S of Reichstein, in the doses used in this study, had no effect on any of the observed clinical, chemical, or hematological manifestations of six classical cases of rheumatoid arthritis.

Received January 12, 1950. P.S.E.B.M., 1950, v73.

Action of Chloromycetin and of Aureomycin on Normal Tissue Cultures. (17645)

P. LÉPINE, G. BARSKI, AND J. MAURIN. (Introduced by J. E. Smadel.)
From the Virus Research Division, Pasteur Institute, Paris, France.

The two new antibiotics, chloramphenicol (Chloromycetin) and aureomycin, which have been shown to be efficacious in the treatment of a number of infectious diseases(1,2), are perhaps of especial interest because of their capacity to inhibit multiplication of rickettsiae and certain viruses. This activity against agents which maintain an obligate intracellular parasitic existence led us to investigate the effect of the antibiotics on the proliferation of normal cells in tissue cultures.

Materials and methods. Tissue culture method. Chick embryo tissues were cultivated on a plastic membrane in a liquid medium by a technique described elsewhere(3,4). Fifteen-day-old embryos were used when the explants were prepared from heart or lung and 11-day embryos when mesencephalon provided the tissue. The liquid medium, containing defibrinated plasma, embryo extract, and Tyrode's solution, was changed on the third day of incubation. Under these conditions of cultivation, the proliferating cells grew in a flat single layer sheet and consisted almost entirely of fibroblasts from the cardiac tissue and of epithelial cells from the lung tissue. The amount of growth of tissue was estimated on the fifth day in the following manner: Using a camera lucida, the margins of the original explant and of the proliferated tissue were outlined on paper of uniform thickness. The pattern of the new growth exclusive of the explant was cut out with scissors. These patterns from the 5 to 16 duplicate cultures in each individual test were weighed and the average value taken as the coefficient of new growth. Explants from the same batch of embryos were used in a given experiment which consisted of control cultures and test cultures containing the designated amounts of antibiotic. The coefficients of growth for the control cultures differed in each experiment despite the care exercised in selecting explants of approximately the same size and shape and in eliminating other variables. Nevertheless, the trend of results obtained in an experiment with varying amounts of antibiotic were reproducible.

Antibiotics. A crystalline preparation of chloramphenicol (Chloromycetin, Lot X 3143), provided by Parke, Davis and Company, and a preparation of aureomycin hydrochloride (Duomycin, Lot 7-8411), provided by Lederle Laboratories, were used in the present work. The drugs, dissolved in physiological saline solution, were added to the liquid media used in the tissue cultures to give the final concentrations desired. All media used in a test, including that for the control cultures, was diluted to the same proportion with saline solution. The presence of chloramphenicol even in concentrations of 1,000 γ/ml had no effect on the pH of the media and caused no precipitate. On the other hand, a slight turbidity was noted in media containing 100 γ/ml of aureomycin and a precipitate in that containing 1,000 γ/ml. Further-

1. Smadel, J. E., *Am. J. Med.*, 1949, v7, 671.
2. Rose, H. M., and Kneeland, Y., Jr., *Am. J. Med.*, 1949, v7, 532.
3. Wirth, J., and Barski, G., *Ann. Inst. Pasteur*, 1947, v73, 987.
4. Barski, G., and Maurin, J., *Ann. Inst. Pasteur*, 1948, v74, 312; *ibid.*, 1949, v76, 295.

more, the presence of these concentrations of aureomycin caused the pH of the media to drop from 7.3-7.4 to slightly below 7.0 but the pH returned to 7.2 when the mixtures were incubated overnight. The bacteriostatic effect of the two antibiotics was tested against the Pasteur Institute strain of *Escherichia coli* under various conditions. The minimal concentration of chloramphenicol which caused complete inhibition of growth of the organism for 24 hours was between 2.5 and 5.0 γ/ml irrespective of whether the substrate was Ringer's solution, Tyrode's solution or the special liquid medium used for tissue cultures. Furthermore, in the tissue culture media the values were the same when the mixture was tested fresh or after storage at 4° or 37°C for 24 hours. Under similar conditions the inhibitory level of aureomycin was 10.0 to 50.0 γ/ml. These findings are in agreement with those of Smith and his coworkers(5) with chloramphenicol but the inhibitory range of aureomycin for the present organism differs somewhat from the observations of Bliss and Chandler(6) with aureomycin on their strain of *E. coli*. The two sets of observations do not agree on the effect of proteins in the media and of temperature on the deterioration of aureomycin. The Baltimore workers who used a different method concluded that the antibacterial effect of this antibiotic was greatly diminished in the presence of serum and by storage at 24° or 37° C. The fluid of the test tissue cultures was replaced on the third day with liquid media containing fresh antibiotic.

Results. Data on the inhibitory effect of various concentrations of chloramphenicol and aureomycin on the growth of fibroblasts and epithelial cells *in vitro* are summarized in Table I. There was a slight but consistent diminution in proliferation of both types of cells in the presence of 10 γ/ml of chloramphenicol. The effect was marked when the concentration of this drug was 100 γ/ml and essentially no growth was noted in the cultures containing 1,000 γ/ml. Microscopic examination of the tissue cells revealed no change in appearance in those grown in 10 γ/ml. However, the cells from the cultures containing 100 γ/ml showed numerous vacuoles and fatty degeneration. Moreover, the proliferating cells no longer grew in a smooth flat sheet. The fibroblasts formed a loose network and the epithelial cells tended to pile up at the thickened peripheral margin. The few fibroblasts and epithelial cells which succeeded in proliferating in the cultures containing 1,000 γ/ml underwent degeneration terminating in death.

The effect of aureomycin on the growth of epithelial cells is of the same order as that of chloramphenicol, see Table I. The data suggest that fibroblasts are more readily inhibited by aureomycin than are epithelial cells.

Similar experiments were made with tissue cultures seeded with tissue from the mesencephalon. The results of these tests, which are summarized in Table II, are in agreement with those in which heart and lung were employed in that concentrations of 100 γ/ml of either antibiotic markedly inhibit cell migration and 1,000 γ/ml prevents it. In this experiment 10 γ/ml of chloramphenicol had an effect whereas the same concentration of aureomycin did not.

Discussion. Concentrations of chloramphenicol which appreciably inhibit *in vitro* proliferation of fibroblasts, epithelial cells and cells from explants of embryonal brain tissue are within the range attained in the blood of patients who receive therapeutic amounts of this antibiotic(7). While less voluminous data are available on the blood levels of aureomycin in patients being treated with this drug(8), the levels approach those which inhibit growth of tissue cells *in vitro*. It may well be that the present observations are not applicable to mature cells or to proliferating

5. Smith, R. M., Joslyn, D. A., Gruhzit, O. M., McLean, I. W., Jr., Penner, M. A., and Ehrlich, J., *J. Bact.*, 1948, v55, 425.

6. Bliss, E. A., and Chandler, C. A., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 467.

7. (a) Smadel, J. E., Woodward, T. E., Ley, H. L., Jr., and Lewthwaite, R., *J. Clin. Invest.*, 1949, v28, 1196; (b) Woodward, T. E., Smadel, J. E., and Ley, H. L., Jr., *J. Clin. Invest.*, 1949, in press.

8. Brainerd, H., Lennette, E. H., Meiklejohn, G., Bruyn, H. B., Jr., and Clark, W. H., *J. Clin. Invest.*, 1949, v28, 992.

TABLE I.
Effect of Chloramphenicol and Aureomycin on Growth of Tissue Cultures.

Chick embryo tissue	Type of cell growth	Antibiotic		Experiment 1		Experiment 2	
		Type	Concentration, γ/ml	Coef. of new growth	No. of cultures	Coef. of new growth	No. of cultures
Heart	Fibroblast	Control	0	20	8	36	12
		Chloramphenicol	10	17	7	28	15
		"	100	8	14	9	15
		"	1000	0.5	14	0	8
		Control	0	63	14		
		Aureomycin	1	53	10		
		"	10	48	11		
		"	100	2	5		
		"	1000	0	10		
Lung	Epithelial	Control	0	17	12	19	15
		Chloramphenicol	10	14	8	11	13
		"	100	10	15	4	16
		"	1000	3	14	—	—
		Control	0	34	11		
		Aureomycin	1	32	15		
		"	10	29	12		
		"	100	22	7		
		"	1000	0	12		

TABLE II.
Effect of Antibiotics on Proliferation of Cells from Explants of Brain.

Antibiotic	Conc., γ/ml	Coef. of new growth	No. of cultures
Control	0	20	12
Chloramphenicol	10	13	12
"	100	12	12
"	1000	0	10
Aureomycin	10	19	11
"	100	8	12
"	1000	0	10

cells in the living host. However, the phenomena are worth considering particularly in patients who require repair of tissue and who are maintained on prolonged therapeutic regimens.

The observations that concentrations of 100 γ/ml of chloramphenicol are directly toxic for cells growing in tissue culture, as shown by histological changes, are in accord with the finding that the LD_{50} of chloramphenicol in 6-day embryonated eggs is approximately 1.5-2.0 mg(9). In the latter instance the over-all concentration of drug in fluids and tissues of the embryo is of the order of 30-40 γ/ml. It may be pointed out that patients with blood levels of 100 γ/ml of chloramphenicol for short periods of time have not shown toxic manifestations(7).

Summary. Proliferating epithelial cells and fibroblasts, and cells from explants of embryonal brain tissue are retarded in growth *in vitro* by concentrations of chloramphenicol and aureomycin of 10 γ/ml and completely inhibited from growing by concentrations of 1,000 γ/ml. The concentrations required for appreciable inhibition of growth of cells are somewhat in excess of those which are bacteriostatic for most susceptible organisms but are within the range attained in the blood of treated patients. The amounts of the antibiotics required to produce toxic changes

9. Jackson, E. B., unpublished data.

in the tissue culture cells are of the order sometimes reached in treating patients but those required for complete suppression of growth are well beyond clinical experience.

Received January 13, 1950. P.S.E.B.M., 1950, v73.

Production of Achondroplasia in the Chick Embryo with Thallium.* (17646)

DAVID A. KARNOFSKY, LOIS P. RIDGWAY, AND PRISCILLA A. PATTERSON.
(Introduced by C. P. Rhoads.)

From the Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.

The "4-amino" derivatives of folic acid have occasionally produced alopecia in man(1). Since thallium is also a systemically-acting depilatory(2,3), is was of interest to determine whether these two agents had other similar biological effects. The "4-amino" derivatives of folic acid produced a marked inhibition of growth of the chick embryo(4). It was, therefore, decided to study the effects of thallium on the same system.

Methods. Fertile White Leghorn eggs were obtained from a commercial source. The eggs were incubated at 38°C and 85% relative humidity. Thallium sulfate was made up in distilled water as a solution containing 10 mg/cc. This solution (the usual volume was 0.05 to 0.20 cc) was injected into the yolk sac of the 4 day embryo, except for a few experiments using 2, 10 and 12 day embryos. The eggs were candled daily, and most of the embryos were sacrificed at 18 to 21 days of age. Some of the embryos were cleared and their skeletons stained with alizarin red S by the method described by Couch, *et al.*(5). The protective value of manganese sulfate, riboflavin, biotin and nicotinamide against thallium was examined by injecting each of these agents separately, but simultaneously, with thallium sulfate into the yolk sac of the 4 day embryo.

Results. The acute (0-4 days after injection) LD_{50} of thallium sulfate for the 4 day embryo is about 1.3 mg/egg. Most of the embryos surviving the acute lethal effect of 1 mg/egg lived to 18 to 22 days of incubation, although none hatched (Table I). Thallium produced a severe and consistent picture of achondroplasia in the chick embryo, which was evident in embryos sacrificed or dying at 10 days of incubation or later. At the larger doses of thallium (1 to 2 mg/egg), achondroplasia was associated with a moderate to severe degree of growth inhibition. At the smaller dose of 0.5 mg/egg, growth inhibition was negligible, although a severe achondroplasia was frequently present. The gross appearance of the affected embryos is shown in Fig. 1. The embryos injected at 4 days of incubation showed a marked degree of achondroplasia, whereas those treated after 10 to 12 days of incubation showed a less severe but definite inhibition in the growth of the extremities. The severely achondroplastic embryos (treated at 4 days) showed very short limbs with feet that were of normal size

* This study was aided by grants from the National Cancer Institute, National Institute of Health, United States Public Health Service, and the American Cancer Society.

1. Burchenal, J. H., Karnofsky, D. A., Southam, C. M., Myers, W. P. L., Craver, L. F., Dargeon, H. W., and Rhoads, C. P., to be published.

2. Heyroth, F. F., *Thallium, a Review and Summary of Medical Literature,* Public Health Reports, Supplement No. 197, 1947.

3. Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics,* 1941, The Macmillan Co., New York.

4. Karnofsky, D. A., Patterson, P. A., and Ridgway, L. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 447.

5. Couch, J. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Anat. Rec.,* 1948, v100, 29.