uronidase was gauged by the disappearance of fluorescein dye injected with it intracutaneously. The failure of antagonism to the action of hyaluronidase to occur in patients with hypoadrenocorticism was noted. The possibility of utilizing the method described as a test for pituitary-adrenal response to epinephrine is discussed.

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Effects of Reichstein's Compound S, Pregnenetriolone Acetate, and 17-Alpha Hydroxyprogesterone in Rheumatoid Arthritis. (17644)

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The promising results of Hench, Kendall, and Polley(1) with 17-hydroxy 11-dehydrocorticosterone (Compound E) in patients with rheumatoid arthritis and more recent ample confirmation(2,3) suggested that other related steroid compounds might also be of value in this disease. The steroids used in this study, 17-Alpha Hydroxy-11-Desoxycorticosterone-21-Acetate (Reichstein's Compound S Acetate), 17-Hydroxyprogesterone (17-Alpha Hydroxyprogesterone), and 4-Pregnene-17-, 20,21-Triol-3-One-20-21-Diacetate (Pregnenetriolone Diacetate) were made available to us by Dr. James A. Shannon, of the National Heart Institute, who also supplied us with their formulation. The compounds were dried and screened and suspended* in a sterile aqueous menstrum containing .25% Tween 80.

Two patients with classical rheumatoid arthritis were treated with each of the above compounds in the dosage of 100 mg daily for 7½ days (750 mg), and 400 mg daily for 4½ days (1750), divided into 6 daily doses and given at 4 hour intervals intramuscularly. All patients received placebo therapy for at least one week before steroid therapy was begun. Metabolic balance studies were not carried out but each patient was placed on a constant diet at least one week before control studies were begun. Serum chlorides, potassium, sodium, carbon dioxide combining power, urea nitrogen, uric acid, and fasting blood sugar determinations were estimated twice during the control period and after each period of therapy. The twenty-four hour urinary excretion of sodium, potassium, urea, chloride, creatinine, and uric acid was estimated in the same fashion. Intravenous glucose tolerance tests were done on all patients during the control period and after therapy. Hemoglobin, hematocrit, sedimentation rate, white blood count, differential, and total eosinophil counts were done twice on all patients during the control period and after each study period.

In addition to the laboratory data, each patient was very carefully followed by the same physician with daily weight, blood pressure, and estimation of degree of swelling and limitation of motion of each involved joint.

Results. In no case was objective improvement of any involved joint observed. Subjective improvement was fleeting and all patients felt worse by the end of the study. In one of the patients treated with Compound S Acetate definite swelling, heat, and tenderness appeared in a previously uninvolved joint during therapy. There was no change in the blood pressure or weight of any of the patients. There were no changes in any of the hematologic or chemical data observed during the course of therapy.

1. Hench, P. S., Kendall, E. E., and Polley, H. F., *Proc. Staff Meet., Mayo Clinic,* 1949, v24, 181.

2. Thorn, G. W., Baylis, T. B., Massell, B. F., Forsham, P. H., Hill, S. R., Jr., Smith, S., and Warren, J. E., *N. Eng. J. Med.,* 1949, v241, 529.

3. Boland, E. W., and Headley, N. E., *J. A. M. A.,* 1949, v141, 301.

* Suspensions made through the courtesy of Hynson, Westcott & Dunning.

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.

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

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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.