has observed that in human skin the perivascular histiocytes in the dermal papillae phagocytize lipids as well as pigment.

Growth of sebaceous glands and sebaceous accumulation is not impeded by biotin deficiency in mice, but normal storage of lipids in the sebaceous cells and the release of sebum is impaired. Growth of the glands is indicated by the presence of intermediate sebaceous cells and by mitotic activity in the peripheral cells (cf. Montagna and Kenyon(15) for the growth of sebaceous glands in the rabbit). This is in accord with the reports of Bosse and Axelrod(16) who find that wound healing in the skin of biotin deficient rats occurs as rapidly as in normal skin. There is an agreement here also with the conclusions of Wilson and Leduc(3) that in animals deficient in biotin "the capacity of tissue cells for mitosis is not impaired", despite a decline in the mitotic activity in the liver during the development of the deficiency. Abnormal functions of the sebaceous glands, however, is indicated by the absence of brown seborrheal incrustation, the scantiness of sudanophilia in the stratum corneum, the absence of birefringent lipids, the failure of the lipids to give a positive Liebermann-Burchard test, and by the fragmentation of the peripheral sebaceous cells.

Summary and Conclusions. In the mouse the growth potentials of the skin and its appendages are not appreciably affected by biotin deficiency, but normal differentiation and function are impaired. There is a slight increase in mitotic activity in the epidermis and in the sebaceous glands. The pilosebaceous units are blocked by keratinized debris and little or no sebum is released upon the surface of the skin. In the sebaceous cells, the stored lipid droplets are much larger than in those of normal animals. Unlike the sebaceous lipids of normal glands, those of biotin deficient mice are isotropic and negative to the Liebermann-Burchard test. There is abundant cellular fragmentation at the periphery of sebaceous glands and the lipids are apparently phagocytized by histiocytes in the dermis. The melanin-bearing cells around the sebaceous cells, when present, also contain lipid droplets. As a rule, neither histiocytes nor melanin-bearing cells contain demonstrable lipids in the skin of normal mice. There is an increase in the number of mesenchymatous cells, mast cells and leukocytic cells in the dermis of biotin deficient mice.

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15. Montagna, W., and Kenyon, P., *Anat. Rec.*, 1949, v103, 665.

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Preliminary Observations on the Antiarthritic Effect of 21-Acetoxypregnenolone.* (17601)

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(Introduced by W. E. Ehrich)

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It has been shown by Seifter, Baeder, and Begany(1) that the permeability of the synovial membrane in rabbits is influenced by enzymes and by steroids. Hyaluronidase and desoxycorticosterone (DOCA) were found to markedly increase permeability, while corti-

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1. Seifter, J., Baeder, D. H., and Begany, A. J., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 277.

sone was the most effective in inhibiting permeability through normal and through hyaluronidase treated membranes. Alarm reaction and administration of adrenocorticotrophic hormone were as effective as cortisone. These data were interpreted to mean that normal permeability of the synovial membrane is maintained by a balance between the opposing actions of the two types of adrenal steroids on the ground substance mucopolysaccharides such as hyaluronic acid. According to this view bacterial infection with release of enzymes such as hyaluronidase, or adaptation disease characterized by excess production of DOCA depolymerize the mucopolysaccharides of the ground substance and permit the formation of edema and swelling of the membrane. Cortisone alters the mucopolysaccharides so that they become more resistant to enzymes and to DOCA, their water binding capacity is decreased, and they become freed from edema.

Seifter, Baeder, and Sollins(2) considered the therapeutic effect of cortisone in arthritis to consist of at least 3 phases: (1) immediate anti-enzyme or anti-DOCA effect on the synovial membrane (end organ) resulting in alleviation of edema and swelling; (2) healing action on synoviae possibly by general effects on carbohydrate metabolism; and (3) general metabolic effect on the rheumatoid state. They employed the rabbit synovial membrane technic to screen more than 20 steroids† and to compare their efficiency with cortisone on the end organ. The barrier effect of cortisone was rated as 100%. On this basis, the barrier of the normal membrane had a 4.5% cortisone rating. All but 2 of the steroids screened decreased permeability of the synovial membrane. Pregnenolone, testosterone, combinations of these, and most of the other steroids had less than 40% cortisone effect. Two steroids were found to be at least as active as cortisone. One of these, 21-acetoxypregnenolone, had activity 130% that of cortisone. It also antagonized the effect of DOCA and hyaluronidase‡ on the synovial membrane. The anti-DOCA effect was five times as great as the anti-hyaluronidase effect. Comparison of the formula of this compound with that of DOCA indicated resemblance of structure sufficient to warrant consideration of its action as a competitive inhibitor of DOCA.

We decided to employ 21-acetoxypregnenolone as an investigative tool to determine whether the simple end organ effect would be sufficient to alleviate rheumatoid arthritis or whether the other actions of cortisone contribute an important role in the treatment of this disease. In this paper we are reporting the preliminary observations which confirm the findings in the rabbit studies. Evaluation of 21-acetoxypregnenolone as a treatment is being carried out on a separate group of hospitalized patients on whom the customary laboratory investigations of the rheumatoid state and necessary metabolic studies are being carried out.

Material and method. Seven patients were selected for this preliminary study according to the following criteria: (1) The patient must have rheumatoid arthritis of a moderate or marked degree, (2) the rheumatic process must be demonstrably active, and (3) the patient must be ambulatory and engaged in his usual activity. One patient, case No. 7, was hospitalized and placed at bed rest. Ambulant cases were selected in an attempt to minimize the known beneficial effects of bed rest. All patients had previously received routine arthritic management. With the exception of case No. 6, each patient was given 100 mg cholesterol daily by intramuscular injection for 3 days prior to the administration of 21-acetoxypregnenolone. 21-Acetoxypregnenolone was then administered intramuscularly, 100 mg the first day, 200 mg on the second day, and 300 mg daily thereafter for a total period of 14 days. At the end of this period the dosage was decreased to 200 mg, and the interval was lengthened to 2 days. Each patient was examined daily by one of us (D.R.F.) and at 3 day intervals by all of

2. Seifter, J., Baeder, D. H., and Sollins, I. V., to be published.

† These compounds were prepared by Doctors John Pataki, Stephen Kaufmann, and George Rosenkranz of the Research Laboratories, Syntex, S.A.

‡ Hydase, Wyeth.

us. Improvement in overall function, swelling, pain, local inflammation, and joint tenderness was measured in accordance with the Criteria for Gauging Rheumatic Improvement as agreed upon by the New York Rheumatism Association: grade O = no improvement; grade + = slight; grade ++ = moderate; grade +++ = marked; grade ++++ = very marked.

Case No. 1, M. H. Age: 39. Occupation: typist. Diagnosis: rheumatoid arthritis. Duration of disease: 2½ years. Joints chiefly involved: both shoulders, and interphalangeal joints of both hands. No change in subjective or objective findings was noted during 3 days of cholesterol administration. Significant improvement first noted on 4th day of 21-acetoxypregnenolone administration. Grade +++ improvement in pain, tenderness, and function noted by all observers on 5th day. Improvement continued over 2 weeks period and patient stated that typing had greatly improved, fatigue had lessened, and sense of well being had improved; improvement was rated as grade ++++. Slight return of swelling and tenderness accompanied reduction of dosage and lengthening of interval. Weight gain of 2 lbs in 2 weeks was noted. No evidence of fluid retention.

Case No. 2, H. B. Age: 34. Occupation: housewife. Diagnosis: rheumatoid arthritis. Duration of disease: 16 years. Joints chiefly involved: both knees, left elbow, and right wrist. Patient reported an aggravation of symptoms during three days of cholesterol therapy. Significant improvement first noted on third day of 21-acetoxypregnenolone administration. Improvement in euphoria, function, pain, tenderness, and swelling was rated as grade +++ on 5th day. Improvement over 2 weeks period was rated as grade ++++ and patient was able to climb stairs for first time in 2 years. Reduction in dosage and lengthening of interval did not cause regression in symptoms. Patient gained 1½ lbs in two weeks period and showed no evidence of fluid retention.

Case No. 3, N.N. Age: 56. Occupation: housewife. Diagnosis: rheumatoid arthritis. Duration of disease: 5 years. Joints chiefly involved: left wrist and left knee. Patient reported aggravation of symptoms during 3 days administration of cholesterol. Significant improvement first noted on 3rd day of 21-acetoxypregnenolone administration. Evaluation on the 5th day revealed remarkable reduction in swelling and grade +++ improvement in local inflammation, tenderness, pain, function, and general mood. By the 8th day patient reported that she had no arthritic symptoms. Evaluation of improvement at end of 2 weeks period revealed grade ++++ improvement in all phases. Reduction in dosage and lengthening of interval was attended by only a slight regression in previously noted improvement. Patient had a total weight gain of 1 lb in two weeks and showed no evidence of fluid retention.

Case No. 4, A. S. Age: 43. Occupation: housewife. Diagnosis: rheumatoid arthritis. Duration of disease: 8 years. Joints chiefly involved: both shoulders, right wrist, right elbow, and metacarpophalangeal joints. This patient was in a severe relapse at the start of treatment and was becoming rapidly worse. Three days of cholesterol therapy seemed to aggravate symptoms. First significant improvement was noted on the 8th day of treatment with 21-acetoxypregnenolone. Evaluation at end of 2 weeks period was rated as grade +++ improvement in general function and in all joints. Reduction in dose and lengthening of interval resulted in a moderate relapse in previously noted improvement. Patient gained no weight during two weeks interval and there was no evidence of fluid retention.

Case No. 5, M. W. Age: 65. Occupation: housewife. Diagnosis: rheumatoid arthritis. Duration of disease: 7 years. Joints chiefly involved: knees, right shoulder, wrists, and metacarpophalangeal joints. Increased fatigue and no improvement in joint symptoms noted during 3 days cholesterol therapy. First significant improvement occurred on 3rd day of treatment with 21-acetoxypregnenolone, with full motion of right shoulder unattended by pain. Improvement in general function, mood, and all joints was rated as grade ++++ on the 5th day of therapy. Improvement continued over two weeks period despite great increase in daily activity. Patient gained 2½ lbs over two weeks period and

there was no evidence of fluid retention. Patient noted a slight decrease in functional ability of right hand on reduction of dosage and increase in interval.

Case No. 6, M.C. Age: 46. Occupation: dietitian. Diagnosis: rheumatoid arthritis. Duration of disease: 38 years. All joints involved. Many joints "burned-out" and ankylosed. Clinical activity most pronounced in right knee. Marked soft tissue swelling in hands and knees with grade II dependent edema in ankles. Patient not given cholesterol but started immediately on 21-acetoxypregnenolone, 300 mg daily. Reduction in swelling noted 48 hours after onset of therapy. Grade ++++ reduction in pain and swelling of right knee noted after 3 days of therapy.

Case No. 7, S. K. Age: 19. Occupation: student. Diagnosis: rheumatoid arthritis. Duration of disease: 3½ years. Joints chiefly involved: knees, metacarpophalangeal joints and interphalangeal joints. This patient was hospitalized for metabolic study, which will be included in a later report. No improvement during 3 days of cholesterol therapy. Significant improvement first noted on 3rd day of administration of 21-acetoxypregnenolone. Evaluation on the 5th day of therapy revealed grade ++++ improvement in swelling, function, and tenderness. Patient noted only minimal stiffness upon arising in morning.

Summary and conclusions. 1. 21-Acetoxypregnenolone was selected for a limited study in 7 ambulatory patients with rheumatoid arthritis because in the rabbit synovial membrane screen it was at least as good as cortisone in decreasing permeability and it showed powerful anti-DOCA action on synovial membrane.

2. The effect on the end organ seen in the screen in rabbits manifested itself in each of the seven patients by reduction in swelling, lessening of the pain, and increased motion in the joints.

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Inotropic Action of Epinephrine, Nor-epinephrine, and N-Isopropyl-Norepinephrine on Heart Muscle.*† (17602)

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In a recent report, Goldenberg *et al.*(1) stated that "nor-epinephrine hypertension is due to an increase of total peripheral resistance, with no significant change, or even a drop in cardiac output whereas epinephrine hypertension is the result of a significant increase of cardiac output." It is not clear which of the factors that determine cardiac output are responsible for the difference of response to epinephrine and nor-epinephrine. One factor which can be evaluated is the effect of the two substances on the contractile force of myocardium. Krop(2) has shown that epinephrine exerts a marked inotropic action on heart muscle, but similar studies have not been reported with nor-epinephrine. Therefore, a comparison was made of the effects of U.S.P. epinephrine, d-l nor-epinephrine (Arterenol) and N-isopropyl-nor-epinephrine (Isuprel) on the contractile force of heart muscle, in an attempt to determine whether the differences in cardiac output reported by Goldenberg *et al.* are due to a lack of inotropic effect by nor-epinephrine.

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2. Krop, S., *J. Pharm. and Exp. Therap.*, 1944, v82, 48.