pathogenic for suckling infants of the C3H inbred line. Cell suspensions (20%) prepared from liver, spleen and lymph glands of leukemic mice of the Ak line, reproduced acute leukemia within 12 days when inoculated under the skin (0.05 cc) of newly born suckling mice of the C3H line.

2. This striking susceptibility of suckling C3H infants to inoculation with Ak leukemia appears to be limited to their first few days of life. Practically all infants of the C3H line (82 out of 85) developed leukemia when inoculated within the first 7 days of life; only 27 out of 57, however, were found to be susceptible when inoculated at ages varying from 8 to 15 days. Of 60 infants, 16 to 30 days old, only 15 reacted to inoculation.

3. Forty-nine adult mice of the C3H line were inoculated subcutaneously and 9 intraperitoneally with large doses (0.2 to 1 cc) of Ak leukemia and none reacted. When, however, overwhelming doses (3 cc each) of the Ak leukemic cell suspension were injected into adult mice of the C3H line, 1 out of 5 inoculated subcutaneously, and all 6 inoculated intraperitoneally died from leukemia.

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Inhibition of Hyaluronidase *in vivo* by Adrenal Cortical Activation. (17643)

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Among the actions of the adrenal steroids is the inhibition of the spreading effect of hyaluronidase injected into the skin(1). We have attempted to measure the decrease in hyaluronidase activity following stimulation of the adrenal cortex in the human. Of the various methods employed to measure hyaluronidase activity, the skin diffusion technic seemed the most practical for wide clinical application. In this study, a dilute preparation of fluorescein was used as an indicator dye for intracutaneous injection with the enzyme. Fluorescein was shown by Bukantz and Dammin(2) to be satisfactory for evaluating the effect of antihistaminic drugs upon the action of histamine. Activation of the adrenal cortex following the administration of epinephrine was established by Vogt(3) and Long(4); the possible magnitude of these responses being studied also by Vogt. The mode of stimulation of the adrenal cortex through the anterior pituitary(4) and, perhaps hypothalamus(5) has been shown. In the present study, epinephrine has been used to stimulate the pituitary-adrenal system in control patients in order to demonstrate inhibition of hyaluronidase injected intracutaneously when the adrenal cortex is activated.

Method. A test for skin diffusion was performed using fluorescein (1:25,000) and hyaluronidase* (150 TRU/cc) drawn into a tuberculin syringe for intracutaneous injection. From 0.01 to 0.03 cc of the enzyme are added to the dye to produce a volume of 0.1 cc which is injected into the volar surface of the forearm. The endpoint for the test is noted when the presence of the dye in the skin cannot be appreciated. It is essential to have either daylight or ultraviolet light in the form of a G.E. Purple X bulb or a Wood's lamp to observe the dye disappearance. Gentle stroking with an alcohol sponge enhances the color and the wheal produced by the injection. The test has been done in the fasting state before and 4 hours after a subcutaneous injection of 0.3 cc of 1:1000 epinephrine. Other dyes have been used in an effort to improve on

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2. Bukantz, S. C., and Dammin, G. J., *Science*, 1948, v107, 224.
3. Vogt, M., *J. Physiol.*, 1944, v103, 317.
4. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.
5. Hume, D. M., *J. Clin. Invest.*, 1949, v28, 797.

* Wyeth, Inc., Hydase, supplied by Dr. George E. Farrar.

the endpoint obtained with fluorescein but none have yet been found. The results obtained with fluorescein can be duplicated by the same observer but experience with it is necessary before the test can be evaluated easily. The use of dermofluorometer or skin microscope may resolve this difficulty.

The epinephrine test described by Forsham, *et al.*(6) was performed on 20 patients who were free of acute or debilitating diseases. In addition, there were 3 cases of suspected hypoadrenocorticism tested, and a number of other patients with chronic diseases, or with acute processes which would constitute a background for adrenocortical activity prior to the test. Several observations were made using an aqueous solution of adrenal extract (Upjohn) intra-arterially to note the effect of a high concentration of steroid in the area supplied by the artery.

Results. The time required for fluorescein to disappear from the skin when injected alone averaged 40 minutes. When hyaluronidase is added to fluorescein the color disappears in 4 minutes with a range from 3 to 7 minutes. In 20 patients tested with fluorescein and hyaluronidase, 4 hours after epinephrine the average disappearance time of the dye had increased from 4 minutes to 21 minutes with a range of 15 to 31 minutes. The results of the fluorescein-hyaluronidase skin tests were found to agree with eosinophile response in this group. The average fall in the total eosinophile count was 68.2%. However there were several instances where the fall in eosinophiles was less than 50%. It will be noted that a significant degree of inhibition of the diffusing effect of hyaluronidase was achieved during a period of epinephrine-induced adrenocortical activity as gauged by the eosinophile response. In three control patients hyaluronidase inhibition occurred without the expected eosinophile response. Previous experience(7) has shown that patients with therapeutic blood levels of salicylates or heparin will inhibit the spreading effect of hyaluronidase. There is reason to suspect that digitalis, tocopherols, and ascorbic acid will do the same. One patient receiving salicylate therapy in the present study also showed inhibition of the enzyme initially to 14 minutes which was doubled following epinephrine. This patient had a 90% fall in eosinophiles at the same time. Ascorbic acid with desoxycorticosterone acetate in four cases slightly inhibited hyaluronidase but did not affect the eosinophile count. Intraarterial adrenal steroid was found to inhibit hyaluronidase in the area supplied by the artery.

Following surgery or other procedures of a stressful nature there was inhibition of the hyaluronidase action similar to or greater than that obtained with epinephrine. This finding was associated with a low eosinophile count and indicated a normal pituitary-adrenal response. In three patients with hypoadrenocorticism with low 17-ketosteroid excretion there was no fall in eosinophiles using the epinephrine test. Nor was there any inhibition of hyaluronidase in these patients as measured by the disappearance of fluorescein injected with it. (Table I)

Discussion. Inhibition of hyaluronidase by the administration of adrenal steroids or by stimulation of the adrenal cortex has been demonstrated by several workers. Opsahl(1) found rapid spread of India ink in adrenalectomized animals and that ACE reduced the spread in these animals. An increase in serum inhibitors of hyaluronidase has been noted by Glick(8) following the administration of ACTH. Seifter *et al.*(9) have found that certain steroids or the serum of animals given alarming stimuli will inhibit hyaluronidase *in vitro* and *in vivo*. The inhibition of the spread of dyes by ACE was originally observed by Menkin(10). By use of the technic described in this paper it has been possible to test the functional response of the adrenal cortex to epinephrine or other stimuli in patients. Stimulation of the adrenal cortex results in inhibition of hyaluronidase activity as measured by

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7. Shuman, C. R., in press.

8. McQuarrie, I., Bank, E. G., Ziegler, M. R., and Wright, W. S., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 555.

9. Seifter, J., Balder, D. H., and Dervinis, A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 136.

10. Menkin, V., *Am. J. Physiol.*, 1940, v129, 691.

TABLE I.
Factors Influencing the Disappearance Time of Fluorescein and Hyaluronidase.

cc	Injected	No. of patients	Disappearance time of dye		Eosinophile fall (avg %)
			Avg (min.)	Range	
0.10	F*	26	40	36-64	
0.09	F + H† 0.01 cc	42	4	3-7	
0.09	F and H 0.01 cc 4 hr after 0.3 cc epinephrine				
	A. Control	20	21.7	16-31	68.2 (3 <50%)
	B. Hypoadrenocorticism	3	4.7	1-8	17.5
0.09	F and H 0.01 cc 20 min. after 1 g ascorbic acid I.V. + DCA I.M.	4	20	15-30	
0.09	F and H 0.01 cc 4 hr after operation	2	25	20-30	90
0.09	F and H 0.01 following intra-arterial adrenal cortical extr.	3	12	10-14	

* F = Fluorescein.
† H = Hyaluronidase.

the disappearance of the indicator dye. The use of this test as a method for screening the integrity of the pituitary-adrenal system is suggested. Since none of the initial disappearance times with hyaluronidase were over 8 minutes it is probable that if the time of dye disappearance after epinephrine is longer than 12 minutes the adrenal cortex is functioning normally. Tentatively, on the basis of our observations in 3 patients, it is suggested that if the dye disappears in less than 12 minutes when injected 4 hours after epinephrine, the adrenal cortex is unrepsonsive. None of the drugs known to inhibit hyaluronidase should be given at the time of or prior to the test.

One factor which needs to be evaluated in the response to intracutaneous hyaluronidase is the age of the patient. Our experience has shown that elderly patients show slower diffusion with hyaluronidase than do the younger patients. Perhaps the substrate, hyaluronic acid, in the skin of older patients is more resistant to hydrolysis than that of younger patients. The state of hydration of the control patients was relatively good. It will be necessary to observe the effect of dehydration and edema upon the disappearance time of the indicator dye in order to evaluate the influence of these factors upon the proposed test. The effect of feeding upon the results of the skin test has not been studied. This may prove to be an advantage over the eosinophile count since there is no reason to anticipate that feeding will in any way inhibit the diffusing action of the enzyme. The influence of a variety of steroids upon hyaluronidase action is being studied by the technic described. The present opinion is that certain steroid hormones inhibit the spreading effect of hyaluronidase by producing a change in the hyaluronate substrate of the tissues. It has not yet been possible to demonstrate how the steroids affect ground substance to block the action of the enzyme. The possibility of direct inhibition of the enzyme by steroid hormones or by serum inhibitor activated by these hormones must be considered. The relationship of the interactions between the steroid hormones and the hyaluronate-hyaluronidase system to the collagen diseases may be a fruitful field for investigation.

Summary. The inhibition of hyaluronidase injected into the skin of patients with normal pituitary-adrenal responses to epinephrine has been observed. The diffusing effect of hyal-

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.

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