mentative effects of very small doses of antigonadotrophic serum which have been shown may be explained.

Summary. It has been shown that the majority of the antigonadotrophic serums of rats and rabbits will augment the effect of a crude sheep pituitary extract upon the uterine weight of immature female test rats when administered in sufficiently small doses. A possible explanation of this phenomenon is discussed.

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Effect of Adrenocorticotrophic Hormone (ACTH) on Serum Hyaluronidase Inhibitor in Rheumatic Fever.* (17781)

ALBERT DORFMAN AND FRANCES E. MOSES

From the Department of Pediatrics, the University of Chicago, Chicago, Ill.

Previous studies have shown that the "non-specific" inhibitor of hyaluronidase is elevated in acute rheumatic fever with a subsequent drop below normal levels(1). A similar rise in acute rheumatic fever was found by Grais and Glick(2). More recently Epstein *et al.*(3) failed to confirm these results claiming that no difference was observed between normal plasma and plasma obtained from patients with acute rheumatic fever. The studies of the latter investigators are open to serious question since oxalated blood was employed in these investigations. It has been demonstrated repeatedly that oxalate markedly decreases the hyaluronidase inhibitory activity of blood probably by precipitation of magnesium(4,5).

The striking effect of adrenocorticotrophic hormone (ACTH) on the course of rheumatic fever suggested that some change might be brought about in the hyaluronidase inhibitor, under the influence of this hormone. The data to be reported were obtained on serial sera of 4 children with chronic rheumatic fever. All patients had been ill for a minimum of 7 months before treatment with ACTH, and had striking objective signs and symptoms of rheumatic activity as evidenced by elevated sedimentation rates, tachycardia, and other signs of myocarditis. They were studied on a metabolic ward for 2 weeks before treatment, during at least one month of treatment, and minimum of 2 weeks after cessation of therapy. Details of clinical and metabolic studies will be published separately. All of these patients showed marked clinical and laboratory evidence of remission of rheumatic disease during the course of therapy. Reactivation followed cessation of therapy in all patients.

Methods. Hyaluronidase inhibitor was determined as previously described by the turbidimetric method(6). This method has been shown to have a coefficient of variation of $\pm$ 14%. All sera were analyzed with and without magnesium in 2 dilutions and each determination was done in duplicate. It has been previously shown that the addition of magnesium markedly enhances inhibitor activity(5). Calculations were made in the manner previously described(6). The enzyme was a partially purified bovine testicular

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hyaluronidase which contained 960 units per mg of nitrogen. The hyaluronidase unit was arbitrary as previously defined(7). Inhibitor activity was expressed as units of hyaluronidase inhibited per ml of serum. All bloods were allowed to clot for approximately one hour and then stored at —20°C. Sedimentation rates were determined by the Wintrobe(8) method and uncorrected values are given in the graph. Eosinophil counts were determined by the method of Randolph (9).

Results. The relationship of hyaluronidase inhibitor to dosage of ACTH, sedimentation rate, and eosinophil counts for a typical patient is shown in Fig. 1. The hyaluronidase inhibitor as measued with or without added magnesium shows a marked drop upon administration of ACTH which roughly parallels the clinical course and drop in sedimentation rate. On cessation of ACTH therapy the reappearance of evidence of clinical activity is accompanied by a rise in the hyaluronidase inhibitor. In 2 other patients, one with rheumatoid arthritis and the other with lupus erythematosis disseminata, identical results were obtained. It has been consistently found that the level of inhibitor drops below the average normal value of children of this age. A series of 14 normal children ages 9-13 gave values of 361 units with added magnesium and 128 without magnesium.

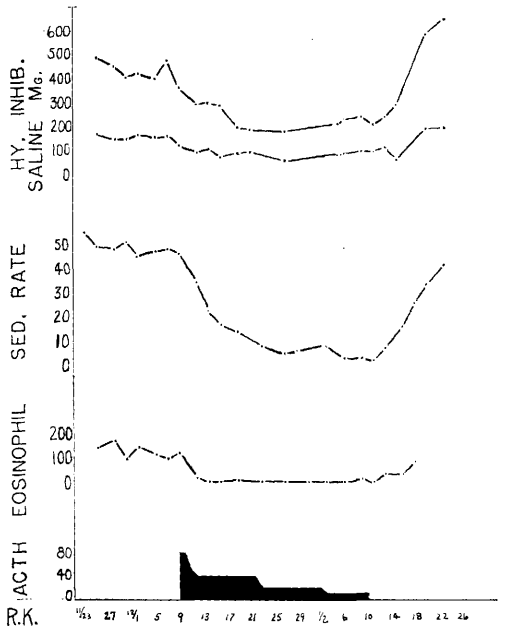

FIG. 1.
Relationship of hyaluronidase inhibitor to sedimentation rate, eosinophil count and ACTH dosage. Other patients studied showed essentially identical results.

Discussion. The significance of the "nonspecific" hyaluronidase inhibitor in the blood of the human is at present unknown. It seems a reasonable assumption that it is not a specific antibody, since all human bloods so far examined inhibit testicular hyaluronidase. The apparent destruction of connective tissue in rheumatic fever and related diseases, the occurrence of hyaluronic acid in connective tissue, and the production of hyaluronidase by certain strains of hemolytic streptococci have led several investigators to speculate as to the possible role of hyaluronidase in these diseases(10-12). The hyaluronidase inhibitor has been considered as a specific protective substance(13). Although it has a specific *in vitro* action there is as yet no evidence that the serum inhibitor is involved in the protection of connective tissue.

Another possible interpretation of the significance of this substance is that the level of hyaluronidase inhibitor may be a reflection of the state of the connective tissue and that the inhibitor may represent some substance which is released on destruction of connective tissue. The decrease in inhibitor under the influence of ACTH (acting by stimulation of adrenal cortical hormone secretion) may be a reflection of change of state of connective tissue brought about by adrenal hormones. The drop of the inhibitor below normal levels may indicate that the connective tissue is

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changed beyond its normal state. Such an interpretation would be consistent with the recent findings by Seifter *et al.*(14), that the alarm reaction in rabbits makes the synovial membrane less susceptible to the action of hyaluronidase. It might then be suggested that cortisone changes the character of connective tissue in such a manner that it no longer reacts to the stimuli which bring about its breakdown in the various diseases of connective tissue. An altered reactivity after ACTH therapy in response to experimental wounds has been shown to occur in rabbits by Ragan *et al.*(15).

Summary. ACTH brings out a marked decrease in the level of the "nonspecific" hyaluronidase inhibitor in patients with rheumatic fever. This decrease parallels the drop in sedimentation rate and clinical course. It is suggested that this may be a reflection of the effect of adrenal hormones on changing the state of connective tissue so that it no longer reacts to the precipitating stimuli in the diseases of connective tissue.

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Renal Glycosuria Induced by Adrenocorticotrophic Hormone.* (17782)

EDWARD H. KASS,† SIDNEY H. INGBAR AND MAXWELL FINLAND

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.

That anterior pituitary hormones will, under suitable conditions, produce temporary or permanent diabetes in the experimental animal is well recognized(1,2). Conn and his co-workers(3) have studied the temporary diabetic state which was induced in normal adult human beings by the continued administration of moderately high doses of pituitary adrenocorticotrophic hormone (ACTH) and have demonstrated that the diminished glucose tolerance and glycosuria which may follow multiple injections of ACTH are associated with diminished blood glutathione levels. Indeed, it was shown that the administration of glutathione will bring about temporary diminution of the glycosuria and hyperglycemia induced by ACTH(4). This finding as Conn *et al.* have pointed out, is of considerable interest in relation to the studies of Lazarow(5) in which it was demonstrated that the effectiveness of alloxan as a diabetogenic agent could likewise be reduced by glutathione and other sulfhydryl-containing compounds.

In the course of studies dealing with the effect of ACTH on acute infectious diseases we have observed, in agreement with Conn *et al.*, that glycosuria could usually be ininduced if sufficiently large doses of ACTH were administered, and that hyperglycemia and impaired glucose tolerance generally accompany the glycosuria. Significant decreases in glutathione levels in the blood have always accompanied these evidences of altered carbohydrate metabolism. It has been suggested that one of the effects of ACTH is to bring about lowering of the "renal threshold" for glucose(3). However, the patients whose

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† Senior Fellow in Virus Diseases, National Research Council.

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