Progonadotrophic Property of Dilute Antigonadotrophic Serums.* (17780)

C. A. Ely,† W. H. McShan, and R. K. Meyer
From the Department of Zoology, University of Wisconsin, Madison

Augmentation of the activity of pituitary gonadotrophic preparations by the blood serum of animals which have been protractedly treated with pituitary gonadotrophins has been observed to occur in the test animal when the 2 substances are simultaneously but separately injected. Ordinarily this property appears in the early stage of the treatment and is eventually replaced by an inhibitory one. It has also been noted that an antiserum may prove to be inhibitory when tested against the pituitary gonadotrophic extract of one species but augmentative with those of one or more other species. Finally, a third phenomenon is recognized wherein some strongly inhibitory serums can be made to exhibit augmentative activity when administered in sufficiently small amounts. With regard to this phenomenon references are to be found in articles by Noble *et al.*(1), Thompson(2), and Katzman, Wade, and Doisy(3).

The present report concerns itself with this latter property as it has been observed under the conditions employed in this laboratory.

Materials and Methods. For the production of the antigonadotrophic substance young sexually mature rabbits and 60-90-day-old male rats of the Sprague-Dawley strain were used. These animals were injected daily subcutaneously with 250-500 mg‡ of crude sheep pituitary extract prepared according to the method of McShan and Meyer(4). At intervals during the injection period these animals were bled from the heart and the serum stored frozen until assays were performed.

Assays were carried out with 21-day-old Sprague-Dawley female rats weighing 40-50 g. Uterine weights were used as indices of activity. A total dose of 5 mg of sheep pituitary extract in saline was given in a series of 6 injections over a period of 3 days with autopsy performed on the morning of the fifth day. The antigonadotrophin was injected at a separate site from the gonadotrophin but concomitantly with it. The amount of antiserum given was varied to suit the purpose of the particular experiment being carried out. However, complete inhibition of 5 mg of pituitary extract was usually obtained with a total dose of .3 ml of antiserum administered in 6 separate injections.

Results and Discussion. As one approach to the study of the progonadotrophic property antigonadotrophic serums were diluted and each dilution assayed separately against a constant amount of pituitary extract. Fig. 1 illustrates the distribution of uterine weights when plotted against the dose of antigonadotrophic serum. Included in this figure are assays made of rat and rabbit blood serum and of rabbit lymph plasma. All antigonadotrophic serums and the lymph plasma were collected from rats or rabbits which had been treated with 250-500 mg of crude sheep pituitary extract daily for periods varying from 80-200 days. The lymph plasma was obtained by cannulation of the thoracic duct of immunized rabbits, and it has been shown to have much the same antigonadotrophic activity as blood serum (Ely, Meyer and McShan)(5). Some few of these serums were

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† Now at the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

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2. Thompson, K. W., Chemistry and Physiology of Hormones, Publ. A.A.A.S., 1944.

3. Katzman, P. A., Wade, N. J., and Doisy, E. A., *Endocrinology*, 1947, v41, 27.

‡ Indicates in all cases milligram equivalents of original pituitary powder used in making the preparation.

4. McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1943, v151, 259.

5. Ely, C. A., Meyer, R. K., and McShan, W. H., *Endocrinology*, 1949, v45, 549.

taken from antihormone animals which had received a single injection of adrenal cortical extract previous to bleeding. Data have been presented (Ely, Meyer and McShan) (5) indicating that this treatment does not markedly alter the antihormone titer. Each point on the figure represents an average of the uterine weights from 2-5 animals with the great majority being averages of those from 3 animals. A total of 329 animals was involved in the assay of 17 different serums. The average uterine weight for 130 controls which received 5 mg of crude sheep pituitary extract only was 45 mg.

It will be noted that with decreased dosage there is an increase in uterine weights which at the lower concentrations is quite marked. Statistical analysis shows that the difference between the mean uterine weight obtained at the .005 ml dose level and the mean uterine weight of controls is highly significant. It should be pointed out, however, that a few of the serums assayed failed to exhibit augmentation at any dilution level. These data have also been included in Fig. 1.

Normal rat and rabbit serum gave no evidence of augmentation when doses ranging from 1.5 ml to .005 ml were assayed by the same technique as that applied to antiserum.

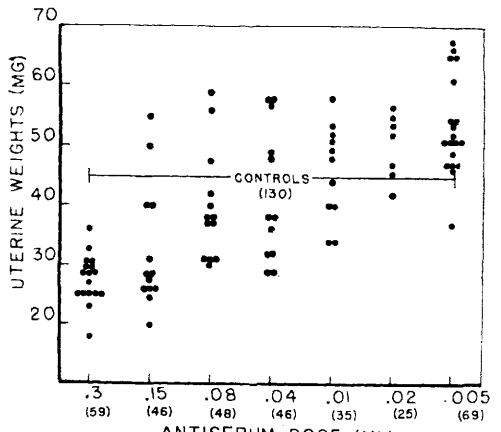

FIG. 1.

Augmentation of the effect of crude sheep pituitary extract by dilute antigonadotrophic serums of rat and rabbit blood serum and rabbit lymph plasma on the immature rat uterus. Numbers in parentheses refer to the total number of assay animals treated with a particular serum dose; each point on the graph represents the average uterine weight of from 2-5 animals assayed at a given time.

It should be emphasized that the capacity of antigonadotrophic serums to become augmentative upon dilution is probably a function of several factors including activity of the preparation injected, length of injection period, and the size of the immunizing dose, and that under different experimental conditions the augmentative effect might be expected to appear at different times after the initial injection and at different dilution levels from those obtained in the study reported here. Several suggestions have been advanced to explain the augmentative activity of serums of animals which have been treated with gonadotrophic materials. These have been discussed by Thompson(6), Rowlands (7), and Rowlands and Williams(8). It is evident, however, that none of these proposals has seemed entirely satisfactory. While a full discussion of the possible interpretation of the phenomenon described here with additional suggestive data is to be taken up elsewhere, it is perhaps worth mentioning that the appearance of augmentation upon dilution may have an immunological explanation. For example it is well known that in certain cases excess antigen in an antigen-antibody system results in the failure of complete precipitation of antibody and may lead to the formation of a soluble antibody-antigen complex. See Kabat and Mayer(9), and Boyd(10). Thus if the assumption is made that upon the introduction of small amounts of antibody (antigonadotrophin) into the test animal an antigen excess is created which results in the formation of soluble antigen-antibody (gonadotrophin-antigonadotrophin) complexes which slowly dissociate releasing small amounts of gonadotrophin, then the aug-

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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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