THE INFLUENCE OF ADRENOCORTICOTROPIC AND GROWTH HORMONES ON ANTIBODY FORMATION*

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There is now much evidence indicating that the administration to experimental animals of high doses of pituitary adrenocorticotropic hormone (ACTH) or of the adrenal glucocorticoids (cortisone or hydrocortisone) during the period of active immunization may result in decreased formation of serum antibodies to the specific antigen employed (1, 2). However, relatively few studies have been carried out on the counteraction of this antibody depression by the simultaneous use of an anabolic hormone such as growth hormone (somatotropin, STH), or on the influence of the latter hormone alone on antibody formation.

The following investigation was carried out in conjunction with studies dealing with the influence of ACTH and STH on resistance to infection. the details of which will be reported in a subsequent publication.

Materials and Methods

Animals.—Young, adult female rats of the Long-Evans strain, 3 to 3½ months of age at the beginning of the experiment and with an average weight of 215 gm. (195–240), were maintained on our standard laboratory diet[1] given *ad libitum*.

Hormones.—Highly purified STH was prepared according to the method previously described (3). It was found to be homogeneous by boundary electrophoresis and ultracentrifugation, as well as according to end-group analyses, zone electrophoresis on starch and adsorption chromatography. A total dose of 10 mg. administered over a period of 4 days produced no histological evidence of the presence of adrenocorticotropic, thyrotropic, or gonadotropic activity in hypophysectomized female rats (operated upon at 28 days of age and injected 14 days postoperatively); a total dose of 5 mg. injected subcutaneously into month old squabs for 4 days gave no lactogenic activity. A dose of 0.1 mg. produced no adrenal ascorbic acid depletion in hypophysectomized rats. Bioassay for melanophore-expanding activity in hypophysectomized *Rana pipiens* disclosed no intermedin contamination

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[1] Composition of the stock diet is as follows: Ground whole wheat, 68.5 per cent, casein, 5 per cent, fish meal, 10 per cent, fish oil, 5 per cent, alfalfa leaf meal, 10 per cent, sodium chloride, 1.5 per cent, KI solution (450 mg. per liter) at 50 mg. per 100 lbs. of diet.

following a dose of 0.2 mg. According to the tibia width assay method, a total dose of 0.08 mg. administered over an interval of 4 days induced an increment of the uncalcified cartilage of the tibia in hypophysectomized rats (operated upon at 28 days of age and injected 14 days postoperatively), amounting to 80 micra over the controls. In similar experimental animals, a daily dose of 0.01 mg. caused an increase of 12 gm. in body weight in 10 days.

The ACTH employed was the F fraction prepared from sheep pituitary glands by procedures involving acid-acetone extraction, NaCl precipitation, oxycellulose adsorption and

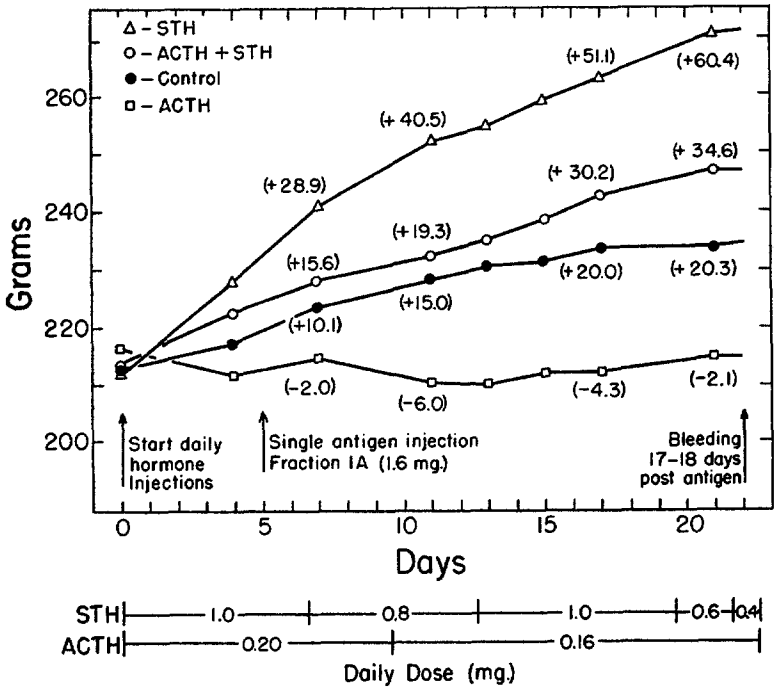

FIG. 1. The influence of ACTH and STH on body weights of normal rats during the period of the formation of antibodies to Fraction IA of *Pasteurella pestis*. Each group is composed of 22 to 23 rats. Weight change in grams is represented by numbers in parentheses

dioxane fractionation (4); it possessed an activity of 25 U.S.P. units per mg. The method of preparation precluded the possibility of contamination with any appreciable amounts of other pituitary hormones at the dose levels employed. A modified Moon assay (5), in which immature male rats were employed for testing adrenal weight-stimulating activity, was performed. The animals (28 day old male rats) were given subcutaneous injections, once daily for 4 days, of 0.05 mg. of ACTH suspended in 0.1 cc. of 5 per cent beeswax (6) in peanut oil. At this dose, a 57 per cent increase in adrenal weight and a 62 per cent involution of the thymus were observed when the animals were sacrificed 20 hours after the last injection.

Antigen.—Fraction IA (7, 8), a soluble protein[2] envelope antigen extracted from *Pasteurella pestis* organisms, was obtained through the courtesy of Drs. K. F. Meyer and T. H.

[2] Fraction IA contains a small amount of polysaccharide.

Chen of the Hooper Foundation for Medical Research, University of California, San Francisco. The antigen was injected with an adjuvant, bayol-arlacel, prepared according to the method given by Cohn (9); this adjuvant is essentially that of Freund, without the incorporation of tubercle bacilli.

Experimental Procedure.—One hundred and twelve rats were uniformly apportioned on the basis of body weight into 5 groups of approximately 22 rats each. The animals in group

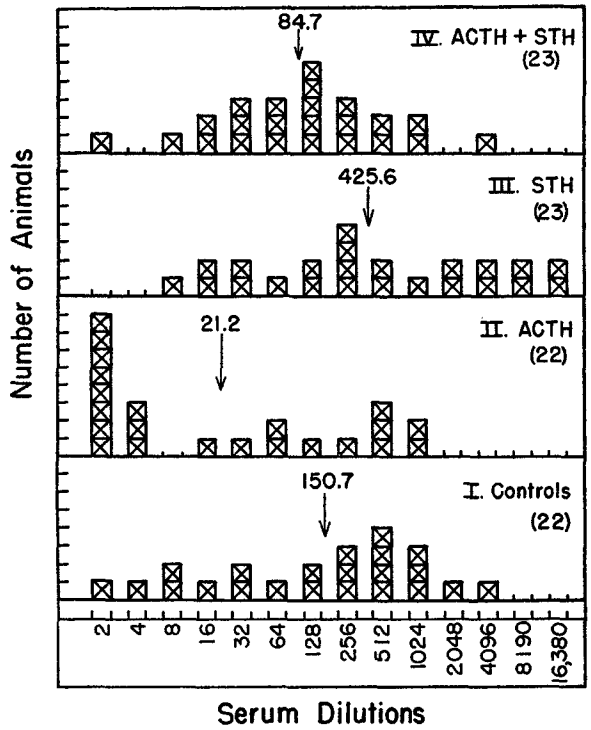

FIG. 2. The influence of the administration of ACTH and STH on serum antibody levels of rats immunized to Fraction IA of *Pasteurella pestis*. The highest serum dilution giving a moderate to strong positive hemagglutination reaction with Fraction IA was considered to be titration end-point. Each cross represents the antibody titer of one animal. Arrows indicate positions of geometric means, determined by obtaining the antilog of the mean log dilutions. Mean log dilutions ± S.E. for the various groups are as follows: Group I, 2.18 ± 0.19; II, 1.33 ± 0.21; III, 2.63 ± 0.20; IV, 1.93 ± 0.17. I *vs.* II = $p < 0.01$; I *vs.* III = $p > 0.05$; I *vs.* IV = $p > 0.20$; II *vs.* IV = $p < 0.05$ [p values from Fisher's table of t].

I served as immunized controls. Those in groups II–IV were also immunized, but received daily hormone injections for a period of 22 days, beginning 5 days prior to the injection of the antigen. Group V was a non-immunized, non-hormone-treated contol. The antigen was given only once, in a total volume of 1.0 cc. containing 1.6 mg. of Fraction IA, of which 0.6 cc. was injected intramuscularly and 0.4 cc. subcutaneously. The daily dose of STH was 1.0 mg., except as otherwise indicated on the graph (Fig. 1), in 0.5 cc. saline. The ACTH, suspended in a vehicle containing 5 per cent beeswax in peanut oil, was injected in a volume

of 0.05–0.1 cc. All injections of hormone were made subcutaneously. Animals were bled by cardiac puncture on the 17th or 18th day after the injection of the antigen. Serum samples were preserved with merthiolate (1:10,000) and stored under refrigeration. The level of serum antibodies to Fraction I A was determined through the use of the highly sensitive hemagglutination method of Boyden (10) as adapted by Chen and Meyer to *Pasteurella pestis* (11). The highest dilution of the antiserum that gave a moderate or strong positive hemagglutination reaction was taken as the titration end-point.

RESULTS

The dose levels of ACTH were just sufficient to suppress the growth of the rats (Fig. 1), whereas the immunized controls gained an average of 20 gm. per rat up to the time of bleeding (21 days). The rats given STH gained an average of 60 gm. per animal while those of the ACTH-STH group gained an average of 35 gm. each over the same interval.

When expressed as the geometric mean (antilog of the mean of the log dilutions), the mean antibody titer of the immunized controls was 1:151 (Fig. 2). ACTH administration caused a rather marked depression of the antibody level, resulting in a mean titer of 1:21, with 50 per cent of the serum samples showing titers of only 1:2 and 1:4. STH alone gave a slightly elevated mean titer of 1:426, with 17 per cent of the samples showing titers that were higher than those in any other group (1:8190 to 1:16,380). The marked depression due to ACTH injections was effectively counteracted by the simultaneous use of STH, so that the average titer of the ACTH-STH group was not significantly different from that of the controls. All of the non-immunized control animals gave negative titers to the antigen employed.

DISCUSSION

Kass, Lundgren, and Finland (12) were unsuccessful in their attempt to counteract with STH the depression of resistance to experimental pneumococcal and viral influenza infection in mice induced by adrenal steroids. They were unable, at the dose levels employed, to obtain a proper balance between cortisone and STH with respect to body weight. It was difficult to obtain any information on the possible mutually counteracting effects of these two hormones with respect to antibody formation, since at the time the serum samples were taken, 6 days after the infection, the antibody levels in both the experimental and control animals were too low.

Hoene, Rindani, and Heuser (13) induced a significant depression of hemolytic antibodies to sheep erythrocytes in rats with daily injections of a relatively high dose of hydrocortisone (2.5 mg.). They were able to obtain a good balance between the doses of hydrocortisone and STH with respect to body and splenic weights. It may be noted, however, that their dosage of STH (4 mg. per day) had to be very high for a rat, in order adequately to offset

certain effects of the hydrocortisone. Strangely, the mean antibody titer resulting from the administration of STH alone was found to be significantly below that observed for the controls. The investigators considered these results as possibly being due to "non-specific effects of STH in the present state of purity." The combination of hydrocortisone and STH resulted in titers somewhat higher than those obtained with the corticoid alone, but the average titer was still considerably below that of the controls.

In our own studies reported here, an effort was made to use no more of the antibody-depressing agent (ACTH) than was sufficient to prevent the normal body weight gain for young adult rats. We found that this dose level of the particular ACTH preparation employed was high enough to cause a significant reduction of antibody formation, and at the same time low enough to avoid the necessity for administering too high a level of STH in order to overcome the suppression of both body weight and antibody formation due to ACTH. It is of interest to note that it has been previously reported that the depressing effect on body weight accompanying relatively high doses of ACTH in normal (14) rats is not due to decreased food consumption. This would suggest that the depressing effect on antibody formation is likewise not mediated by any decrease in food consumption. It may be due, rather, to the decreased rate of protein synthesis, a view shared by many investigators, for example those in reference 15.

SUMMARY

The influence of pituitary adrenocorticotropic hormone (ACTH) and growth hormone (somatotropin, STH), singly and in combination, has been studied in normal, young adult rats, with respect to antibody formation against Fraction IA of *Pasteurella pestis*.

When ACTH was administered during the period of immunization, in a daily dose just sufficient to prevent body weight increase relative to the non-treated, immunized controls, serum antibody levels against the specific antigen employed were significantly depressed.

The administration of STH alone resulted in a marked increase in body weight. The increase in antibody level was not significant at the 5 per cent level when compared with the control values.

The same dosage of STH given simultaneously with ACTH maintained body weight at a level slightly above that of the controls, and resulted in an effective counteraction of the antibody depression produced by the latter hormone.

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