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Immunologic Studies of Prolactin.* (26290)

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Although the capacity of prolactin to induce antibody formation has been demonstrated(1), no recent studies have been reported in which the antigenicity of relatively small doses of more purified prolactin preparations have been evaluated. A study was therefore designed to evaluate the antigenicity of a relatively pure preparation of prolactin and to investigate the possibility that such antibodies could be employed in a useful assay for this hormone. The present study confirmed the ability of prolactin to induce antibody formation. The antisera that were obtained yielded hemagglutination reactions which were inhibited by small quantities of added prolactin, but which were not inhibited by much larger amounts of other pituitary hormones.

Materials and methods. Prolactin: A highly purified preparation of ovine lactogenic hormone with a stated potency of 15 I.U./mg† was used. *Immunization.* Adult white rabbits were injected subcutaneously with saline solutions of prolactin suspended in equal volumes of Freund's complete adjuvant. Each of the following amounts of prolactin was given to a different animal: 0.1 mg (Rabbit 1), 2.5 mg (Rabbit 2), 12.5 mg (Rabbit 3). Eleven weeks later boosters of 0.25 mg of prolactin were given to rabbits 2 and 3, and one week subsequent to initial injection, a 0.5 mg booster was given to rabbit 1.‡ *Sampling*: Peripheral blood samples were obtained from all 3 animals just prior to immunization. Rabbit 1 was bled again 3 weeks later, while multiple samples were obtained from Rabbits 2 and 3 from the third to 12th week after the initial immunization. The antiserum obtained on the 12th week from Rabbit 2 was used in evaluation of the inhibition of hemagglutination. *Hemagglutination titers*: All serum samples were heated to 56°C for 30 minutes to inactivate complement and absorbed with sheep erythrocytes to prevent nonspecific hemagglutination. Antigen-antibody reaction was evaluated by the hemagglutination technic(2). Each 1.0 ml volume of a 2.5% suspension of washed, tannic acid treated sheep erythrocytes was sensitized with 0.2 mg of prolactin. Of this suspension 0.06 ml was added to tubes containing either normal rabbit serum, serial dilutions of antisera, or serial dilutions of test materials in antiserum. Significant hemagglutination was considered to have occurred if at the end of 3 hours the erythrocyte suspension appeared as a granular layer of cells covering the bottom of the tube. Less marked agglutinations were classified as negative.

Results. All animals lacked demonstrable anti-prolactin antibodies prior to immunization; all developed such antibodies after im-

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‡ Boosters for Rabbits 2 and 3 were in saline. Booster for Rabbit 1 was in Freund's complete adjuvant.

TABLE I. Inhibition of Prolactin-Anti-Prolactin Hemagglutination.

Substance	Quantity, μg	Inhibition*
Prolactin†	.026	+
Crude extract, ovine pituitary	.1	+
Somatotropin, bovine	7.8	+
Corticotrophin, commercial	60.0	—
TSH, purified	5.0	—
TSH, Armour, commercial	250.0	—
Human albumin, commercial	"	—
Human immune globulin, commercial	"	—
LH	"	—
FSH	"	—
HGG	"	—

* + values indicate smallest amount found to inhibit the reaction. — values indicate minimum amount of substances which failed to inhibit hemagglutination; larger quantities were not tested.

† Prolactin—original antigen; TSH, purified—highly purified bovine thyrotropin, 5 units/mg, prepared by Dr. John Pierce; LH, FSH, HGG—luteinizing hormone, follicle stimulating hormone and human chorionic gonadotropin, respectively, supplied by the Endocrine Study Section, Nat. Inst. Health, U.S.P.H.S.

munization. Qualitative "ring" tests showed them to be precipitable; quantitative hemagglutination assays showed that Rabbbit 3, which was given the largest dose of antigen, had the highest antibody titer in each of the post-immunization bleedings.

The specificity of the hemagglutination caused by the reaction of anti-prolactin antiserum with prolactin-sensitized erythrocytes was evaluated by determining the amount of test substances added to antiserum that inhibited hemagglutination. Significant inhibition was considered to have occurred if there was at least a 4-fold difference between antiserum without inhibitor and an equal volume of antiserum with added inhibitory material. Each experiment was performed at least twice before the findings were assumed to be valid. Table I summarizes the data obtained.

Discussion. The antigenicity of ovine prolactin in rabbits confirmed older studies; the ability of as little as 0.1 mg of a purified preparation to induce antibody formation raised the hope that this reaction may have been specific for prolactin. Although *in vitro* cross-reaction was found with small amounts of bovine growth hormone, it should be noted that 300 times more growth hormone than the prolactin was required for inhibition of hemagglutination. Furthermore, bioassay of the growth hormone for prolactin revealed significant contamination.§ A variety of other proteins, including several of the pituitary hormones failed to cross-react. Thus, the present data are consistent with the possibility that the antibodies produced against prolactin were relatively specific.

Recently Simkin and Goodhart, using the pigeon crop bioassay, have reported the presence of prolactin in the blood of human subjects(3). The assay system used in the present study is capable of detecting as little as 0.026 μg of prolactin. If anti-ovine prolactin antiserum cross-reacts with human prolactin, or if potent and specific antisera can be prepared against human prolactin, then an immuno-assay could be developed that would be sufficiently sensitive to evaluate the prolactin content of human blood.

Summary. Rabbits immunized with relatively pure prolactin developed antibodies against this hormone. The antiserum seemed to be specific for prolactin, and could be used in an assay system capable of detecting as little as 0.026 μg (0.4 milliunits) of prolactin.

§ Reported by Endocrine Study Section, Nat. Inst. of Health.

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