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Immunologic Inhibition of Endogenous or Exogenous Thyrotropin in Various Species.* (29281)

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Thyrotropin antiserums prepared in rabbits or guinea pigs neutralize the biologic activity of exogenous thyrotropin (TSH) in mice(1-4). This effect occurs after preincubation of TSH and antiserum prior to injection or following separate injections of each. The present study was undertaken to determine the immunobiologic effect of thyrotropin antiserums on either endogenous or exogenous TSH in guinea pigs, rats and mice.

Materials and methods. All animals, except for one group of Swiss Webster mice, were obtained from Charles River Laboratories, Brookline, Mass., and were kept on a diet of Purina laboratory chow and tap water. Supplementary fresh vegetables were given to rabbits and guinea pigs. All animals were weighed regularly. Antiserums for passive administration were prepared in adult rabbits and were treated as described previously(5).

TSH bioassay in mice: A modification of the method of Querido, Kassenaar and Lameyer(6) was used to assay TSH. White female mice weighing 15 to 20 g were divided into groups of 10 animals. On day one, each animal was given intraperitoneally 80 μg of sodium L-thyroxine pentahydrate (equivalent to 69.8 μg L-thyroxine). On day 3, solutions for assay were injected intraperitoneally in 2 separate doses 8 to 10 hours apart. On the morning of day 4, a tracer dose of 0.05 μc of I-131 (0.25 μc/100 g) was given intraperitoneally. This dose produced approximately 1×10^5 cpm in a well scintillation counter and radiation analyzer using a 5 volt window at the major I-131 peak. Eight hours later all animals were killed by ether asphyxiation, the thyroid glands excised, dissolved in sodium hydroxide and counted. The data obtained from assays in 2 strains of mice were plotted as a log dose-response curve using USP Thyrotropin Reference Standard in a range of 5 to 75 mU.†

Endogenous thyrotropin studies: These studies were based upon the proposition that the goitrogenic effect of propylthiouracil results from increased endogenous secretion of TSH; and that this effect of endogenous TSH could be inhibited in mice, rats or guinea pigs by administration of antiserum to bovine TSH if species cross-reactivity existed. Each animal was given subcutaneously a daily dose of 50 mg/kg of an alkaline solution of propylthiouracil (PTU) for 10 days. For guinea pigs, rats and mice, daily doses of antiserum were given intraperitoneally at the same time as the PTU and discontinued on the 10th day. Each animal received a dose

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† All units referred to in this study are based upon USP Reference Standard Thyrotropin of U. S. Pharmacopoea.

of antiserum calculated to be in excess of that required to neutralize 25 mU TSH in mice, adjusted for the weight of that species compared to mice. Different lots of antiserums have different biologic neutralizing capacities which do not necessarily parallel their serologic titer. For this reason, the biologic neutralizing dose (BND) was determined for each lot of antiserum. The BND is defined as the minimum volume (ml) of a particular antiserum which completely neutralizes the biologic activity of a known quantity of USP Thyrotropin Reference Standard using the modified Querido assay. Control groups were injected with either physiologic saline, PTU alone, normal rabbit serum (NRS), or PTU and NRS. Forty-eight hours after discontinuance of injections, a tracer dose of I-131 (0.25 μc/100 g) was administered intraperitoneally to each animal. Eighteen hour later all animals were killed by ether asphyxiation. The thyroid glands were excised, dissolved in sodium hydroxide and counted.

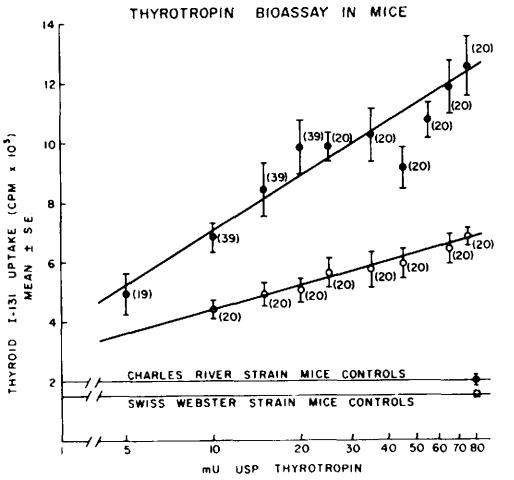

FIG. 1. Log dose-response curves for USP Reference Standard TSH using the modified Querido assay in mice. Mean thyroid counts ± standard error represent thyroidal accumulation 8 hr after administration of I-131. Numbers in parentheses beside mean points indicate no. of animals. Upper curve is that obtained with Charles River C-D mice; lower curve, that of Swiss-Webster mice.

Exogenous thyrotropin studies: These studies were based on previous observations in this laboratory that thyrotropin antisera will neutralize the biologic activity of exogenous thyrotropin in mice(1,4,5). Intact guinea pigs and rats were injected intraperitoneally on day one with 400 μg/100 g of sodium L-thyroxine pentahydrate. Hypophysectomized rats were also used but were not thyroxinized. Thyrotropin solutions made from USP Thyrotropin Reference Standard were incubated with appropriate amounts of antiserum at 37°C for 30 minutes and then kept at 4°C overnight. In these studies, the dosage of antiserum was calculated from the BND of the antiserum and the weight of the animal compared to the weight of assay mice. The precipitates formed were removed by centrifugation, and the supernatant solutions were injected intraperitoneally starting on day 3 in 3 equal doses of 125 mU TSH/100 g animal over 48 hours. In groups in which TSH and antiserum were injected at separate sites, the antiserum was given 12 hours before the first dose of TSH. Control groups were given either physiologic saline, TSH alone, or TSH with NRS. Two to three hours after the last injection, all animals received 0.25 μc/100 g of I-131. Eighteen hours later, the animals were killed and the thyroid glands removed and counted. Hypophysectomy, judged at autopsy by visual inspection, was found to be complete in all rats.

Results. Bio-assay of TSH: The log dose-response relationship for USP Thyrotropin Reference Standard as determined by the modified Querido assay is shown in Fig. 1 for the Charles River C-D and Swiss Webster strains of mice. This response curve appears to be linear in both strains for the dose range 5 to 75 mU. The Charles River C-D mice showed a greater sensitivity to TSH and were used throughout this study. Good reproducibility was obtained most consistently with doses below 40 mU and 25 mU TSH was selected for a standard stimulatory dose and for determination of the BND of various antisera. Efforts to improve the sensitivity of the assay by use of a low iodine diet prior to assay were disappointing in that the responses were more variable for each dose and were less reproducible.

Endogenous TSH: The neutralizing effect of passively administered rabbit antiserum to bovine TSH was shown in guinea pigs, rats and mice (Table I). In these studies there

TABLE I. Inhibition of Guinea Pig, Rat and Mouse Endogenous TSH by Rabbit Antiserum to Bovine TSH.

Species	NSS	PTU	NRS	PTU + NRS	PTU + anti-TSH
Guinea pigs (5)					
Thyroid wt*	15.6 ± 1.1	27.9 ± 2.2	17.5 ± 1.4	29.3 ± 2.3 p <.005‡	20.4 ± .4 p <.01§
Thyroid I-131 counts†	18.4 ± 4.6	38.0 ± 1.4	20.7 ± 2.2	35.7 ± 2.6 p <.025	24.9 ± 2.6 p <.025
Rats (10)					
Thyroid wt*	8.2 ± .4	18.0 ± 1.3	6.3 ± .2	18.7 ± 1.9 p <.001	9.6 ± .5 p <.001
Thyroid I-131 counts†	5.3 ± .2	12.8 ± 1.1	7.8 ± .6	10.7 ± .2 p <.001	6.8 ± .6 p <.001
Mice (10)					
Thyroid wt*	1.7 ± .01	1.9 ± .1	1.6 ± .01	2.2 ± .01 p <.005	1.9 ± .01 p <.05
Thyroid I-131 counts†	2.5 ± .4	6.5 ± 1.3	2.6 ± .3	6.1 ± .8 p <.005	2.5 ± .2 p <.001

Abbreviations: NSS = physiologic saline; PTU = propylthiouracil; NRS = normal rabbit serum; and Anti-TSH = rabbit antiserum to bovine TSH. Numbers in parentheses indicate No. of animals in each group. Values are means ± standard error.

* mg/100 g body wt.

† Counts per min $\times 10^3$.

‡ p values in this column are between PTU + NRS groups compared to NSS groups.

§ p values in this column are between PTU + anti-TSH groups compared to PTU + NRS groups.

was inhibition of both goitrogenesis and radioiodine uptake. Degree of inhibition varied but was significant in each group. Variations in the goitrogenic response to PTU are due, at least in part, to technical difficulties in excising and weighing individual thyroid glands. These results indicate a biologic cross reactivity between rabbit antibodies to bovine TSH and endogenously secreted TSH of guinea pigs, rats and mice.

Exogenous TSH: The effects of passively administered rabbit antiserum to bovine TSH on exogenously administered bovine TSH are tabulated in Table II for thyroxinized guinea pigs and for thyroxinized or hypophysectomized rats. Because of the low dosage and short duration of TSH administration, no significant effect on goitrogenesis was noted. However, there was a definite increase in thyroidal radioiodine uptake following TSH. Passive administration of antiserum produced inhibition of the TSH-induced increase in radioiodine uptakes in these animals. This action was present when antiserum was first preincubated with TSH or when antiserum and TSH were injected separately. In hypophysectomized rats, antiserum added *in vitro* or given by injection resulted in a significant reduction in thyroid weights as well as thyroidal I-131 uptakes.

Discussion. A reliable bioassay procedure for thyrotropin is a critical requisite for correlation with immunologic neutralization. The assay of Querido, Kassenaar and Lameyer was chosen despite its relative insensitivity. It was noted that the sensitivity of this assay as originally reported was derived from the *provisional* USP Thyrotropin Standard which was 20 times more potent than the current USP Thyrotropin Reference Standard(7). A log dose-response curve for the range of 5 to 75 mU of TSH based upon the current USP Reference Standard could not be found in the literature. This relationship was therefore examined in 2 strains of mice and is shown in Fig. 1. A dose of 25 mU produces a satisfactory response which is reasonably precise and reproducible. This dose was selected to determine the biologic neutralizing dose (BND) of different logs of antiserums. Standardization of the biologic activity of various antisera was required since such activity does not necessarily parallel the serologic titer(4).

TABLE II. Inhibition of Exogenous Bovine TSH by Rabbit Antiserum to Bovine TSH in Guinea Pigs and Rats.

Species	NSS	TSH	TSH + NRS	TSH + anti-TSH *In vitro**	TSH + anti-TSH *In vivo*†
Thyroxinized guinea pigs (5)					
Thyroid wt‡	29.9 ± 2.2	34.8 ± 1.3	33.6 ± 3.2	33.1 ± 2.1	34.5 ± 1.9
Thyroid I-131 counts§	10.3 ± 3.0	75.1 ±14.1	84.1 ± 9.1	8.9 ± 2.0	12.4 ± 2.2
Thyroxinized rats (6)					
Thyroid wt‡	8.4 ± .6	9.7 ± .3	9.3 ± .4	10.9 ± .9	11.3 ± .7
Thyroid I-131 counts§	1.2 ± .3	5.3 ± 1.2	5.0 ± .5	1.4 ± .01	1.5 ± .4
Hypophysectomized rats (5)					
Thyroid wt‡	10.0 ± .5	11.1 ± 1.1	12.3 ± .9	8.6 ± .6	7.6 ± .5
Thyroid I-131 counts§	1.8 ± .4	18.2 ± 3.7	26.3 ± 3.8	2.0 ± .2	3.1 ± 1.3

Abbreviations: NSS = physiologic saline; TSH = bovine thyrotropin; NRS = normal rabbit serum; and Anti-TSH = rabbit antiserum to bovine TSH. Numbers in parentheses indicate No. of animals in each group. Values are means ± standard error. Significant changes in thyroid weight occurred only in hypophysectomized rats (TSH + NRS *vs* TSH + anti-TSH *in vitro*: $p < .01$; TSH + NRS *vs* TSH + anti-TSH *in vivo*: $p < .005$).

* TSH and antiserum mixture incubated prior to injection.

† Antiserum injected 12 hr prior to the first dose of TSH.

‡ In mg/100 g body wt.

§ As counts per min × 10^3.

Neutralization of the biologic activity of pituitary and nonpituitary hormones by hormone-directed antiserum has long been known. This subject has been reviewed by Thompson(8) and by Leathem(9). Renewed interest in the antigenicity of protein and polypeptide hormones has confirmed and extended knowledge of the biologic neutralizing capacities of such antisera when passively administered. The current status of these investigations has been summarized(10).

In guinea pigs, rats and mice, passive administration of rabbit antiserum to bovine TSH resulted in neutralization of the biologic activity of endogenous TSH produced by PTU administration. Biologic neutralization of endogenous TSH was achieved when the dose of antiserum was the same as, or greater than, that calculated from the biologic neutralizing dose (BND) of antiserum and the weight of the experimental animal. In preliminary studies, smaller quantities of antiserum failed to neutralize completely endogenous TSH in mice and rats regardless of serologic titer.

These findings in rats are in agreement with reports of others. Baschieri, Negri, de Luca, Sereno and Cramarossa(11) found a definite decrease in thyroid weight and I-131 uptake of rats treated with methylthiouracil and rabbit antiserum to bovine TSH. Reichlin and Boshans(12), using I-131 release curves to demonstrate TSH activity, showed inhibition of I-131 release from rat thyroids following injection of rabbit antiserum to bovine TSH. In our studies and in those of Reichlin and Boshans, comparable doses of antiserums were employed. Baschieri and associates were able to obtain similar neutralizing effects with low doses of antiserum.

In thyroxinized guinea pigs, mice and intact, as well as hypophysectomized rats, passive administration of rabbit antiserum to bovine TSH neutralized the biologic activity of bovine TSH. This effect was shown when the antiserum was first incubated with TSH prior to injection or when it was allowed to circulate in the animal prior to injection of TSH. This effect has previously been demonstrated in mice(1-4). Thus, as would be anticipated, antibodies to bovine TSH neutralize TSH of bovine origin regardless of the species in which this reaction is tested.

The present studies show that rabbit antibodies to bovine TSH biologically neutralize endogenous TSH of rats, guinea pigs and mice. This suggests a molecular similarity of TSH in these species. The cross reactivity of antisera to bovine TSH with human TSH is not clear. Earlier observations demonstrating neutralization of human pituitary thyrotropic fractions by antisera to bovine pitui-

tary TSH(2) have not been confirmed(13, 14). However, the capacity of antisera to bovine TSH to neutralize human TSH may not be an all or none reaction but may be dependent upon quantitative relationships between antigen and antibody. This has been suggested by the reports of Werner and Tierney(15) and of Levy, McGuire and Heideman(16). This important relationship now being investigated is particularly germane to development of immunoassay methods to measure human TSH.

Summary. A modification of the Querido bio-assay for thyrotropin is presented along with log dose-response curves for USP Reference Standard Thyrotropin in 2 different strains of mice. The immunobiologic effects of antisera to bovine thyrotropin (TSH) on either endogenous or exogenous TSH were determined in guinea pigs, intact or hypophysectomized rats and mice. In thyroxinized guinea pigs and thyroxinized intact or hypophysectomized rats, passively administered rabbit antiserum to bovine TSH neutralized the biologic activity of exogenous bovine TSH. Rabbit antiserum passively administered to guinea pigs, rats or mice inhibited the effects of propylthiouracil (PTU) on goitrogenesis and on radioactive iodine uptake, indicating biologic neutralization of endogenous TSH in these species. These findings suggest a molecular similarity of guinea pig, rat and mouse thyrotropin.

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Inhibition of Established Tuberculin Hypersensitivity by Methotrexate. (29282)

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The induction of immune responses in various animal species is inhibited by the antifol methotrexate (aminomethylpteroylglutamic acid(1,2). This drug-induced depression of immune responses has been most thoroughly investigated in guinea pigs in which doses of methotrexate which caused no obvious systemic toxicity suppressed the development of delayed hypersensitivity, antibody production, and the specific febrile response to purified protein antigens(2). Methotrexate treatment has also inhibited the development of experimental allergic encephalomyelitis(3), autoimmune thyroiditis(4), and the homograft response in guinea pigs(5).

Preliminary studies of the mechanism of action of methotrexate on the immune responses of guinea pigs suggested that the drug

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