Quantitative Studies of the Reaction of Bovine Thyrotropin Antiserum with Human and Bovine Thyrotropin Preparations.* (27590)

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Immunochemical technics have been introduced as sensitive assays for insulin and glucagon. The present work, initiated as one of the steps in evaluating the potential of applying such methods to the assay of thyrotropin, showed qualitative similarities and quantitative differences in the immunological properties of human and bovine thyrotropin (TSH). A rabbit was immunized with a purified bovine TSH preparation. The anti-TSH specificity of the resulting antibodies and the quantitative aspects of their cross-reaction with a variety of bovine and human TSH preparations were studied. A hemagglutination technic was used to indicate over-all antigen-antibody reaction, and 2 different bioassays were employed to confirm the presence of antibodies capable of neutralizing the biological action of TSH and to quantitate their affinity for TSH from the two species tested.

Materials and methods. TSH preparations. Two different crude human pituitary preparations were compared with several bovine preparations. They are described in Table I. Samples of the "Pierce" material were labeled with I^{131}, using a micro-distillation technic. Assuming a molecular weight of 28,000 for this material (as if homogeneous) (1), it was estimated that 2.3 iodine atoms were introduced per molecule in the preparation tested. The I^{131}-labeled material had a biological potency equal to another sample that was handled similarly except for iodination.

Preparation of antiserum. A total of 1.2 mg of "Bates" TSH suspended in Freund's complete adjuvant(2) was given in divided doses to a white New Zealand rabbit during a 69-day period. The serum sample used for the present studies was obtained on the 76th day after immunization was begun.

Hemagglutination tests. Previously described methods were used(3). The erythrocytes were sensitized with 10 μg of "Bates" bovine TSH per 1.0 ml of a 2.5% suspension of tanned cells. Failure of antiserum when premixed with a test substance to agglutinate the TSH-sensitized erythrocytes was interpreted as evidence of a significant inhibition reaction.

Bioassay procedures. Both the *in vitro* bovine thyroid slice method of Bakke, Heideman, Lawrence, and Wiberg(4) and the *in vivo* mouse assay of McKenzie(5) were used, with U.S.P. TSH as the standard. Samples and incubation media for the *in vitro* assay contained 0.025% human serum albumin and 0.001 M propylthiouracil. Samples for the *in vivo* assay were diluted to 0.5 ml with phosphate-buffered isotonic saline solution, pH 7.2, also containing 0.025% human serum albumin. Normal rabbit serum in quantities equal to the largest amounts of antiserum used in neutralization studies was without effect. Dilutions of the various TSH preparations and antiserum were mixed and stored overnight at 4°C prior to bioassay. Decrease in the potency of the TSH was evidence of its neutralization by the antiserum. The smallest amount of antiserum that completely abolished the effect of a given TSH preparation and the largest amount of antiserum that failed to alter the response to TSH significantly are shown as limits of the range of the antiserum's neutralizing ability. Although the neutralization range was computed from the mean potency of each preparation, extension of the ranges by incorporating the 95% confidence limits still resulted in no overlap between species and did not alter qualitative conclusions.

Results. The effects of different TSH preparations in inhibiting "TSH - anti-

* Supported in part by grants from U.S.P.H.S. and Veterans Administration.

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TABLE I. Thyrotropin Preparations Used in Immunologic Evaluation and Their Inhibition of TSH-Anti-TSH Hemagglutination.*

Preparation	Description	Potency (95% confidence limits), USP units/mg	Threshold of inhibition†	
			μg	mu TSH
U.S.P.	Bovine	.074		
Armour‡	Bovine, similar to Thyrotropar®	.69 (.41 - 1.2)	.090	.062
"Bates"‡	Bovine, prepared by Condliffe and Bates(6)	10‖	.030	.30
"Pierce"§	Bovine, prepared by Pierce and Carsten(1)	19 (16 -23)	.07	1.3
I^{131}-"Pierce"§	Bovine, "Pierce" with an estimated 2.3 iodine atoms per molecule	19 (16 -23)¶		
Human, 1‡	Prepared by Ciereszko's method, CA fraction(7)	.053 (.031- .089)	26	1.4
" , 2§	Prepared by method of Heideman *et al.*, excluding chromatography (8)	.058 (.047- .071)		

* The authors are grateful to Armour Laboratories, Dr. Robert W. Bates, and Dr. John G. Pierce for their generosity in providing samples of bovine TSH.
† Erythrocytes sensitized with "Bates" TSH. Antiserum: anti-"Bates."
‡ Assayed by *in vivo* mouse assay(5).
§ Assayed by *in vitro* thyroid slice assay(4).
‖ Nominal potency supplied.
¶ Corrected for losses in dialysis and handling.

TSH" hemagglutination are listed in Table I. It was apparent that the inhibition reaction was not proportional to the TSH content. For example, 0.062 mu (0.037-0.11 mu)‡ of Armour TSH caused the same degree of inhibition as 1.3 mu (1.1-1.6 mu)‡ of the "Pierce" preparation. It required 1.4 mu (0.80-2.9 mu)‡ of a crude human TSH preparation to cause the same degree of inhibition. As much as 100 μg of commercial serum albumin or human γ-globulin (poliomyelitis immune globulin) was without effect.

The effect of the antiserum on biological activity of TSH is represented in Fig. 1. The 2 assay methods gave similar results. Although varying considerably in their purity, all the bovine preparations required similar amounts of antiserum per unit to neutralize their biological activity. The human material required considerably more antiserum to neutralize its biological activity. The bovine USP standard and the human preparations were of similar potency; nevertheless, about 20 times more antiserum was required to neutralize the latter. The I^{131}-labeled bovine material was approximately equivalent to its unlabeled precursor in its reaction with antiserum.

Discussion. The present study demonstrates the shortcomings of the hemagglutination reaction as applied to the assay of TSH when a heterogeneous antigen was used; inhibition was not proportional to the biological activity of the various bovine preparations. Neutralization of the biological activity of TSH by an antiserum is evidence of a specific effect. The present data, showing

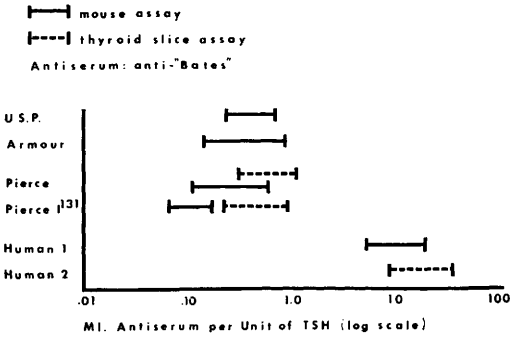

FIG. 1. Neutralization of TSH bioactivity by anti-TSH serum. Ranges of neutralization were computed from mean potencies of each preparation and amounts of antiserum which either completely neutralized or failed to affect the response of a given preparation.

‡95% confidence limits.

that bovine TSH preparations of varying degrees of purity were neutralized by similar amounts of an anti-bovine TSH serum per unit of TSH activity, support this specificity. Qualitative data reported by others have indicated neutralization of the biological activity of both human and bovine TSH preparations by anti-bovine TSH sera(9,10,11). The quantitative data of the present studies, with agreement between two bioassay methods using markedly different indices of response, demonstrated that considerably more anti-bovine TSH serum was required to neutralize the biological effect of human TSH than that of bovine TSH. This fact suggests structural differences in the hormones themselves or their association with other species-specific proteins present in the preparations (*cf.* Dedman, Fawcett, and Morris(12)).

The biological activity of purified bovine TSH and that of an I^{131}-labeled derivative of the same material were neutralized by similar amounts of antiserum. Thus the iodinated preparation maintained its immunologic integrity, as well as its biological activity.

Summary. Two kinds of immunological comparisons between several bovine and human preparations of thyrotropin (TSH), including an I^{131}-labeled bovine preparation, were made with the use of a rabbit antiserum against a purified bovine preparation. Using erythrocytes sensitized with a sample of the immunizing antigen, the inhibition of hemagglutination demonstrated cross-reaction between a crude human TSH preparation and anti-bovine TSH serum, but degree of inhibition was not proportional to biological activity when 3 bovine preparations were compared. Both the *in vivo* mouse I^{131} release and the *in vitro* bovine thyroid slice bioassay showed that the antiserum neutralized TSH. Results from the 2 assays were in agreement. Crude human TSH preparations required considerably more antiserum per unit of biological activity than did either crude or purified bovine TSH preparations. The labeled bovine preparation retained both biological potency and immunological integrity.

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Received May 11, 1962. P.S.E.B.M., 1962, v110.

An Antitussive Property of Diphenhydramine in Dogs. (27591)

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The clinical popularity of expectorant mixtures containing diphenhydramine (Benadryl®) hydrochloride has usually been ascribed to this agent's primary antihistaminic action and its secondary sedative, anticholinergic and local anesthetic properties. Examination of the drug for an outright antitussive effect was long delayed, but in 1960 we eventually completed an experiment in dogs to test for this property. Independently and almost simultaneously, H. A. Bickerman (personal communication) has found that 50 mg diphenhydramine hydrochloride orally in man is roughly equivalent to 15 mg codeine