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Effects of Antiserum in Bioassay of Thyrotropin and Thyroid Activator of Hyperthyroidism. (26032)

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Recently evidence has accrued that there is in the blood of patients with Graves' disease a substance which can stimulate the thyroid gland of guinea pig(1,2), mouse(3), and presumably the patient's own gland. It has been suggested that this substance may not be thyrotropin of pituitary origin(4); this was based on several qualitative differences shown to exist between the activity found in the blood in hyperthyroidism and that found either in pituitary extracts or in the blood of patients with myxedema. A further distinguishing feature is furnished in the present study, wherein antisera to bovine (pituitary) thyrotropin, produced in rabbits, were found to inhibit thyrotropic activity of bovine pituitary extracts and that in the blood of patients with myxedema, but not the thyroid activator in serum of patients with hyperthyroidism.

Methods. Antiserum (A) to commercial bovine thyrotropin (thyrotron, Nordic Biochemicals) was prepared by administering intramuscularly, 3 weeks apart, 2 injections of 2.5 mg of this preparation emulsified in Freund's adjuvant (complete), to totally thyroidectomized rabbits. Antiserum (B) to U.S.P. standard (bovine) thyrotropin was prepared by administering intradermally 5 weekly injections of 1 mg of this preparation emulsified in Freund's adjuvant (complete), in the interdigital webs of intact rabbits. These antisera were tested for titer and specificity using the bis-diazotized benzidine hemagglutination test, as previously described (5,6,7).

Thyrotropic effect was assayed by a method previously described(8); mice with thyroidal iodine labelled with I^{131} were injected intravenously with serum or other test solution and response measured as an increase in circulating radio-activity. This was expressed as a percentage of pre-injection concentration of I^{131} in the blood and a positive response was thus greater than 100%. Four to 6 mice were injected with each test solution. Thyrotropin of pituitary extracts or that found in the blood in myxedema produced maximal effect within 2 hours, but the thyroid activator in the blood in hyperthyroidism had maximal effect only after about 9 hours(3).

Human serum for assay was obtained from 3 patients with spontaneous myxedema and 3 with hyperthyroidism (Graves' disease). Diagnosis had been made previously by conventional criteria of physical examination and measurement of protein bound iodine in serum and thyroid uptake of I^{131}. Serum was separated from the blood within an hour of its being obtained, and stored at 4°C until used for assay, within one week in all instances.

Mixtures of serum or thyrotropin standard (U.S.P.) with antiserum or control serum were kept at room temperature for 30 minutes then centrifuged. Although no gross precipitate was seen the upper portion of fluid in the centrifuge tube was always used for assay.

Results. The hemagglutinating antibody titer of these thyrotropin antisera ranged

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TABLE I. Inhibition of Standard (Pituitary) Thyrotropin by Antiserum.

Exp.		Preparation assayed*	Ratio	Vol of inj. (ml)	Response§
I.	a.	Antiserum A + saline†	1:1	.5	213 ± 97
	b.	" " + .5 mu thyrotropin in saline	1:1	.5	376 ± 73
	c.	Human serum (normal) + .5 mu thyrotropin in saline	1:1	.5	333 ± 13
	d.	" " " + saline	1:1	.5	60 ± 6
Comment:		Antiserum A contained antithyrotropic activity (b less than a + c) simultaneously with thyrotropic activity.			
II.	a.	Antiserum A + .5 mu thyrotropin in saline	1:10	.5	107 ± 39
	b.	" " + .5 mu *idem*	1:100	.5	246 ± 84
	c.	" " + .5 mu "	1:1000	.5	293 ± 88
Comment:		Thyrotropic effect diluted out by 10 parts saline, but antithyrotropic activity retained; latter diluted out by 100 parts saline.			
III.	a.	.5 mu thyrotropin in saline	—	.25	452 ±182
	b.	Antiserum A + .5 mu thyrotropin in saline	1:10	.25	119 ± 39
	c.	N.R.S.‡ + .5 mu *idem*	1:10	.25	399 ± 92
	d.	Antiserum A + 2.0 mu "	1:10	.25	264 ± 75
Comment:		.5 mu thyrotropin inhibited by antiserum diluted 1:10; 2.0 mu thyrotropin partially inhibited by antiserum diluted 1:10 (assuming that 2.0 mu should have produced a greater response than .5 mu(8)).			
IV.	a.	.25 ml N.R.S. inj. i.v. before .5 mu thyrotropin in .2 ml saline	—	—	240 ± 27
	b.	.25 ml antiserum B inj. i.v. before .5 mu thyrotropin in .2 ml saline	—	—	80 ± 28
Comment:		Inj. of antiserum B inhibited effect of a subsequently inj. dose of thyrotropin.			

Thyrotropin was assayed as described under *Methods*. Control (*i.e.* inactive) injections gave a response of 97 ± 4 (stand. dev.) (8).

* See methods. † Saline = .9% (w/v) sodium chloride in water. ‡ N.R.S. = normal rabbit serum. § Mean ± stand. dev. of responses in 4 to 6 mice in each instance.

from 1/4000 to 1/12,000. Both antisera showed extensive cross reactions with other pituitary hormones, namely, corticotropin, growth hormone and gonadotropin. However, a hundred-fold increment in quantity of any of these hormones, as compared with either thyrotropin preparation, was required to produce a definite antigen-antibody reaction (hemagglutination-inhibition). Both thyrotropin preparations had similar quantitative and qualitative properties in this respect.

Antiserum A was found to contain both thyrotropic and antithyrotropic activity (Exp. I, Table I); only the latter remained in evidence when one volume of antiserum was diluted with 10 volumes of physiological saline. With further dilution (1:100) neither antithyrotropic nor thyrotropic effect was detected (Exp. II, Table I). No thyrotropic effect was shown with antiserum B. Although precise quantitation was not attempted it seems from Exp. III (Table I) that 0.023 ml of antiserum A could inactivate from 0.05 to 0.2 mu. of thyrotropin. In Exp. IV, (Table I) injection of the two substances separately still resulted in inhibition of thyrotropic effect.

With serum from patients with myxedema, endogenous thyrotropic activity was completely inhibited by prior incubation with antiserum added in a ratio of 1 to 10 volumes of human serum (Fig. 1, a). Similar assays carried out with serum from patients with hyperthyroidism (Fig. 1, B) failed to show significant inhibition of the thyroid activator contained therein. In the case of the first 2 sera from hyperthyroid patients 1 part of antiserum was added to 10 parts of human serum; in the third assay (responses 1300 and 1290) antiserum B and human serum were mixed in equal parts.

Discussion. Antisera to commercial and U.S.P. standard thyrotropin preparations had similar immunologic properties. While neither antiserum was specific for thyrotropin, the quantitative serological data suggest that the major antibody in both was probably to

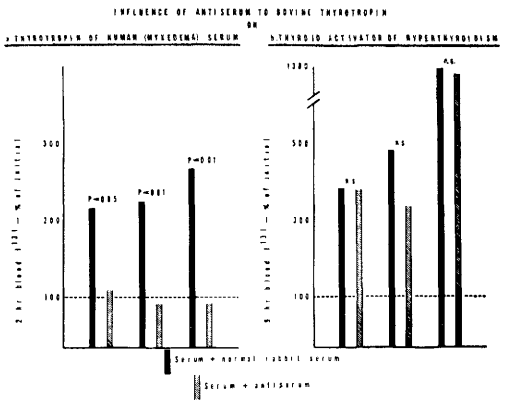

FIG. 1. Horizontal interrupted lines indicate mean values obtained with control studies(3,8). Quantities of normal rabbit serum used in the experiments, when assayed separately, gave negative responses both 2 hr and 9 hr after inj. Significance of the difference of mean responses was established by Student's "t" test.

thyrotropin itself. The cross reactions between thyrotropin antisera and the other pituitary hormones doubtless reflect the impurity of the thyrotropin preparations.

These antisera were shown to inhibit the biological effect of thyrotropin and this was brought about either by preincubation of antiserum with thyrotropin or by separate injection of the two substances. This is in agreement with work reported many years ago(9) and more recently(10,11). Inhibition of thyrotropin in the serum of patients with myxedema by the antisera infers a degree of immunochemical similarity between bovine thyrotropin and human thyrotropin; this was shown also in similar studies using human pituitary extract(11). That the thyroid activator in the blood of the 3 patients with hyperthyroidism was not inhibited infers, on the other hand, that this substance is qualitatively different from thyrotropin. This would be in agreement with other qualitative differences previously described(4).

The findings with serum from patients with Graves' disease are, however, at variance with those reported by Werner and his colleagues (11). They described that the globulin fraction from rabbit antiserum to thyrotropin inhibited thyrotropic activity of an extract of serum from a patient with Graves' disease. The assay method used was similar to our own but response in the mice was measured only 2 hours after injection of the extract. This is considered to be the time at which maximal effect from thyrotropin is found, distinct from the time (7 to 9 hours) after which maximal effect from the thyroid activator of hyperthyroidism is found(3).

It is of interest that thyrotropic and antithyrotropic activity occurred simultaneously in antiserum A. In view of the concentration of thyrotropin reported(12) in normal rabbits, detection of the hormone in blood of thyroidectomized rabbits would be expected with the assay method used here(8). Perhaps this endogenous hormone was combined with antibody, and the complex, retaining thyrotropic activity, remained dissolved in presence of excess antibody.

Summary. Rabbit antisera to commercial and U.S.P. standard thyrotropin preparations were shown to inhibit biological assay of bovine pituitary thyrotropin. While similar inhibition of endogenous thyrotropin in serum from patients with myxedema was shown, the antisera did not inhibit the thyroid activator in blood of patients with Graves' disease. This constitutes further evidence for the dissimilarity of the thyroid activator of hyperthyroidism and thyrotropin.

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