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COMPARATIVE EFFECTS OF IMMUNIZATION OF RABBITS WITH HUMAN THYROGLOBULIN AND HUMAN AND RABBIT THYROID MICROSOMAL FRACTIONS

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SUMMARY

Dutch rabbits were immunized with human thyroglobulin and human and rabbit thyroid microsomal fractions. The animals were bled at intervals and their serum was assayed for thyroid-stimulating activity, thyroglobulin haemagglutinins, and total and free thyroxine (T_4). Their thyroidal radio-iodine uptake and thyroid histology was also studied. Five out of ten animals immunized with human thyroglobulin developed histological evidence of thyroiditis but none had thyroid-stimulating activity in the serum. Only one out of twenty-six animals immunized with rabbit or human thyroid microsomal fractions had any histological abnormality in the thyroid, but eight had significant amounts of thyroid-stimulating activity in their serum. Although the frequency of the latter response was no greater after immunization with rabbit as compared to human thyroid microsomal fraction, there was a significant increase in serum free T_4 in the group immunized with rabbit tissue.

Thyroiditis and rabbit immunologic thyroid stimulator (RITS) formation appear to be completely separable phenomena, and RITS is not a by-product of thyroiditis. The absence of thyroid-stimulating activity in the serum of rabbits immunized with thyroglobulin is further evidence that thyroglobulin is not the important antigen leading to the production of this thyroid-stimulating globulin.

INTRODUCTION

Because of our interest in the possible immunological nature of the long-acting thyroid stimulator (LATS), we immunized rabbits with thyroid microsomal fractions. Many of these animals subsequently developed thyroid-stimulating activity in their serum (Beall & Solomon, 1968). Similar results have been reported by others (Pinchera, Liberti & Badalamenti, 1966; McKenzie, 1968). We have further shown that this thyroid-stimulating globulin has many characteristics of an antibody and that it can be clearly differentiated

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from thyroid-stimulating hormone (TSH) (Solomon & Beall, 1968; Solomon & Beall, 1970). In our laboratory we have referred to this thyroid-stimulating activity in the serum of immunized rabbits with the acronym RITS (rabbit immunologic thyroid stimulator). Animals with RITS activity do not usually appear to have thyroid hyperactivity, although we have found elevated free thyroxine (T_4) values in a few rabbits who had been immunized repeatedly (Solomon & Beall, 1970) and McKenzie (1968) found thyroid function was less easily suppressed by T_4 in a few similarly immunized rabbits than in controls.

Because we thought it possible that a species closer to the human might more easily develop an experimental model of human Graves' disease, we immunized a group of baboons with human thyroid fractions. This procedure produced a severe thyroiditis, together with the appearance of large amounts of TSH but no RITS in the serum (Beall *et al.*, 1969a). This interesting difference in response to similar immunization procedures in the two species suggested the present investigation. We postulated that the antigen responsible for the production of thyroid stimulator was related to the plasma membrane of the thyroid epithelial cell and hence would appear in thyroid microsomal fractions when the tissue was homogenized with a blendor employing a knife blade. It was thought that immunization with purified human thyroglobulin would not result in production of RITS, since we had previously amassed evidence that thyroglobulin is not the material in thyroid extracts which inhibits LATS *in vitro* (Beall *et al.*, 1969b). Finally, we sought to test whether the responses to xenogenic (human) and allogeneic (rabbit) thyroid microsomal fractions would differ.

To test these hypotheses animals were immunized either with human or rabbit thyroid microsomal fractions or with human thyroglobulin.

MATERIALS AND METHODS

Animals

Dutch rabbits of both sexes were used. Although allegedly similar in age, their variability in size belied that contention. The range of weights was 1·27 to 2·84 kg. Animals were assigned to treatment groups in such a way as to minimize the variation in sex, weight and initial thyroid radio-iodine uptake (RAIU). In each group the sexes were evenly distributed, the mean weight ranged from 1·83 to 1·97 kg and the mean radio-iodine uptake to the thyroid (RAIU) varied from 7·2 to 8·5%.

Immunization

Materials. Human thyroglobulin (HTg) was prepared by ammonium sulphate fractionation (Derrien, Michel & Roche, 1946) followed by passage through Sephadex G-200. The material excluded from Sephadex G-200 was dialysed against phosphate-buffered saline (PBS) (0·14 M NaCl+0·01 M sodium phosphate, pH 7·1) and frozen until used. Rabbit thyroid microsomal fraction (RTM) was prepared from the thyroid glands of freshly killed New Zealand white rabbits (packed on ice and shipped unfrozen by air from Pel-Freez Biologicals, Inc., Rogers, Arkansas). Human thyroid microsomal fraction (HTM) was obtained from a pool of normal thyroid tissue obtained at autopsy. The microsomal fraction was prepared in the manner previously described (Beall & Solomon, 1966).

Procedure. Nine animals were immunized with RTM, and seventeen with HTM. One gram-equivalent (g-eq) of HTM or RTM was suspended in 0·5 ml PBS plus 0·5 ml Freund's

complete adjuvant. The emulsion was injected into five subcutaneous sites on the back. 14 days later another one g-eq in 0·5 ml PBS plus 0·5 ml Freund's complete adjuvant was injected into two subcutaneous sites on the back. 4 weeks after the initial immunization, a course of intraperitoneal injections was given. One-half g-eq suspended in 0·5 ml of PBS was administered six times during the next 2 weeks. 7 weeks after the initial injection, RAIU was measured and the animals were bled. During the 8th to 10th week after the initial immunization, the intraperitoneal course of booster injections was repeated. RAIU was again measured during the 11th week and the animals were bled. They were subsequently bled again and sacrificed 12 weeks after the initial immunization. Seven of the seventeen animals immunized with HTM received tri-iodothyronine (T_3) 15 μg s.c. daily through all 12 weeks of the study. Responses of these animals were not different from the ten who did not receive T_3, except that thyroid function data indicated suppression of thyroid function. These data are not included in this report, but the immunological and bioassay data on these seven animals have been pooled with the other ten receiving HTM.

Ten animals were immunized with human thyroglobulin. 15 mg were injected s.c. on days 1, 14, 37, 38, 39 and 40. 15 mg were given i.v. on day 41 and the first bleeding followed 1 week later. A booster series of injections of 15 mg s.c. were given on days 64, 65, 66 and 67. Another 15 mg were given i.v. on day 69. The animals were again bled during the 11th and 12th weeks.

Thyroid stimulating activity

The sera were heated, absorbed with sheep red blood cells and the globulin fraction was obtained by ammonium sulphate precipitation (Beall & Solomon, 1968). The dialysed globulin fraction was then assayed for thyroid-stimulating activity in mice by a modification of the McKenzie technique. Each assay consisted of injections of 0·5 ml of test material into each of six appropriately prepared mice. The increase in blood radioactivity 2 hr after injection was expressed as a percentage of the zero-hour value. This 2-hr response corrected for the response of a control group which received 2% bovine serum albumin was termed a response index and was used as the measure of thyroid stimulation and RITS activity.

Antibody detection methods

Haemagglutinating antibodies to HTg were assayed with the tanned red cell (TRC) method (Fulthorpe *et al.*, 1961). Antibodies to thyroid cytoplasmic and colloid antigens were sought using both unfixed and methanol-fixed frozen sections of thyrotoxic human thyroid. Deposition of rabbit globulins was detected with a fluorescein isothiocyanate-labelled anti-rabbit γ-globulin prepared in goats (Antibodies Incorporated, Davis, California).

Thyroid histology

At the 12th week the animals were sacrificed and the thyroid glands removed. A portion of each of the thyroid lobes was fixed in Bouin's solution, mounted, sectioned serially and stained with haematoxylin and eosin. The slides were coded so that the microscopist was unable to identify the source of the individual thyroid. The code was not broken until all of the sections had been examined and a judgment had been made as to the presence or absence of thyroiditis in the section. Thyroiditis was judged to be present when collections of small round cells were discovered in the thyroid (Weigle, 1965).

Thyroid ^{131}I uptake

Radio-iodine uptake to the thyroid gland (RAIU) was estimated as described previously (Beall *et al.*, 1968) except that in this study the counts were obtained at 27 rather than at 24 hr. Counts were also made at other time intervals in order to learn if maximal thyroid accumulation of ^{131}I occurred in 27 hr. Maximal accumulation occurred as earlier time intervals in a few animals but the values at 27 hr were unchanged. In three animals thyroid ^{131}I accumulation continued to increase progressively over a period of several days. We did not investigate this bizarre phenomenon but speculated that it indicated reingestion of excreted radio-iodine by these animals.

Serum T_4

Serum total T_4 was measured by the 1965 modification of the method of Murphy and Pattee (Murphy & Pattee, 1964; Murphy, 1965). The results are expressed as total T_4 in μg/100 ml and were not corrected for loss in the ethanol–butanol extraction from serum. This loss averaged 23% in control studies.

Serum free T_4

The dialysable fraction of T_4 was measured as described previously (Solomon & Beall, 1970), using a modification of the methods of Schussler & Plager (1967) and Sterling & Brenner (1966).

RESULTS

Half the rabbits (five out of ten) immunized with HTg showed histological evidence of thyroiditis (Table 1). In striking contrast, only one out of twenty-six animals immunized with either RTM or HTM had an alteration of thyroid histology ($P<0{\cdot}01$ using chi squared (χ^2) test). In the one RTM animal whose thyroid was abnormal, the lesion was a

TABLE 1. Incidence of thyroiditis and of RITS after immunization of rabbits

Material injected	N	Thyroiditis	Significant RITS in serum*
HTg	10	5†	0‡
Total TM	26	1†	8‡
RTM	9	1	3
HTM	17	0	5

* Number of animals whose post-immunization serum gave a 2-hr response index significantly greater ($P < 0{\cdot}05$ by t-test) than their pre-immunization control serum in the McKenzie assay.

† $P < 0{\cdot}01$ (χ^2 analysis of HTg vs RTM plus HTM).

‡ $0{\cdot}1 > P > 0{\cdot}05$ (χ^2 analysis of RTM plus HTM vs HTg).

cyst surrounded by fibrosis and cellular infiltration. This was markedly different in appearance from the small foci of lymphocytic infiltration found in the animals immunized with HTg.

Rabbits immunized with HTg did not produce RITS activity (Table 1). Significant amounts of RITS activity were found, however, in the sera of eight out of twenty-six rabbits immunized with rabbit or human thyroid microsomal fractions. This difference between the production of RITS by the HTg and TM groups fell just short of statistical significance ($P<0{\cdot}1>0{\cdot}05$ by χ^2 test).

The differences in RITS production by the various groups are more convincing when each animal's RITS assay value is compared to his pre-immunization value (Table 2). The mean 2-hr response index (%) of serum of the HTg-immunized group declined slightly after immunization while the mean 2-hr response index (%) of the HTM group increased by $25{\cdot}2\pm7{\cdot}7$. The serum of RTM-immunized animals also caused an increase in response index of $9{\cdot}8\pm6{\cdot}3$. Thyroid stimulation by the serum of both the RTM and HTM groups was significantly greater than that of the HTg group (Table 2).

TABLE 2. RITS activity in serum after immunization (7 weeks)

Material injected	N	Change in 2-hr response index from pre-immunization control (mean ± SEM)	P*
HTg	10	$-9{\cdot}9\pm5{\cdot}6$	—
RTM	9	$+9{\cdot}8\pm6{\cdot}3$	$<0{\cdot}05$
HTM	17	$+25{\cdot}2\pm7{\cdot}7$	$<0{\cdot}01$

* For t-test vs HTg group.

Of the rabbits immunized with HTM while receiving T_3, two had significant amounts of thyroid-stimulating activity in their post-immunization serum. The incidence was similar (three out of ten) in the HTM group which did not receive T_3, so these results are pooled in Tables 1 and 2.

Haemagglutinating anti-thyroglobulin antibodies appeared in the serum of all animals. Titres were nearly maximal in 7 weeks in both the HTM and HTg groups. Titres were similar in both groups in all the bleedings (Fig. 1). Haemagglutinating titres to human thyroglobulin in the serum of animals immunized with RTM were significantly less at each bleeding than those of the animals immunized with human antigens. This difference in anti-thyroglobulin response was also detected by colloid immunofluorescence (Table 3). Antibodies to human thyroid cytoplasmic antigens were detected in similar titres in all three groups after immunization.

Total T_4 concentration in serum was frequently elevated after immunization. This phenomenon in rodents who receive thyroid materials has been described by several groups of investigators (Premachandra *et al.*, 1963; McKenzie, 1968; Beall & Solomon, 1968). Although this occurs with great regularity, the wide variability in the degree of this phenomenon is interesting. Extremely high total T_4 concentrations (17·4 and 18·8 μg/100 ml) were found in two of the animals in the HTM group in this study. These animals did not

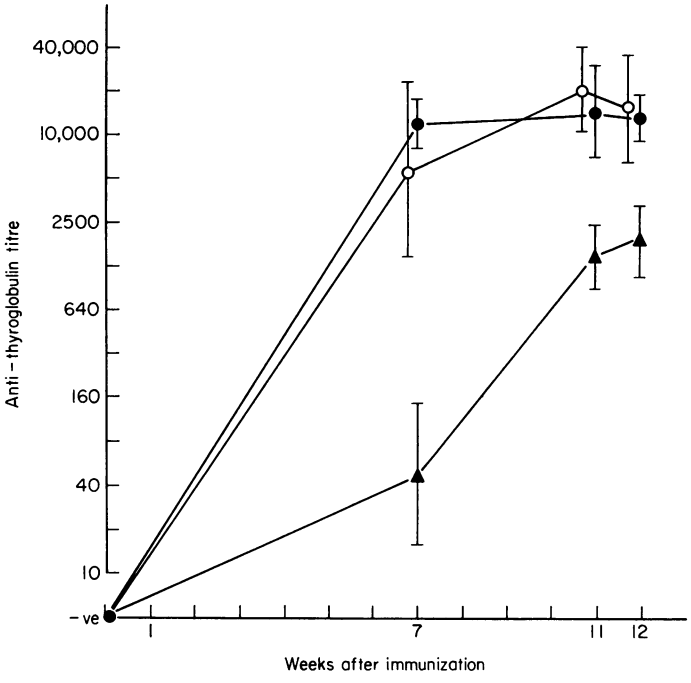

FIG. 1. The titre of haemagglutinating antibodies to human thyroglobulin is shown as the mean ± SEM for each immunization group: human thyroglobulin (HTg) (○); human thyroid microsomes (HTM) (●); and rabbit thyroid microsomes RTM) (▲).

TABLE 3. Immunofluorescence titres of rabbit serum after immunization (11th week), expressed as $\log_{10}$ (Mean ± SEM)

Material injected	N	Thyroid	
		Cytoplasm	Colloid
HTg	10	1·1 ± 0·3	1·7 ± 0·3*
RTM	9	0·8 ± 0·3	0·3 ± 0·2
HTM	9	1·3 ± 0·5	1·7 ± 0·4*

* $P < 0{\cdot}001$ (t-test vs RTM).

have thyroiditis. They had significant RITS activity in serum, and their anti-thyroglobulin titres were among the highest observed.

Serum free T_4 concentration rose significantly in the RTM-immunized group. This change was unrelated to the development of RITS; the only change in RAIU was a transient fall (Table 4). Three RTM-immunized animals did have RITS activity in their serum, but these particular animals had normal free T_4 levels. RAIU increased slightly in the HTM-immunized group.

TABLE 4. Measurements of thyroid function in immunized rabbits (Mean ± SEM)

Group	N	Pre-immunization	Post-immunization	
			7 weeks	11 weeks
Total T_4 μg/100 ml				
HTG	10	3·1±0·1	3·9±0·2*	4·2±0·3*
RTM	9	3·3±0·2	3·9±0·2	4·6±0·4*
HTM	10	3·3±0·2	6·1±1·2*	8·8±2·8
Free T_4 ng/100 ml				
HTG	10	1·7±0·1	2·0±0·1	2·1±0·2
RTM	9	1·7±0·1	2·1±0·1*	2·3±0·2*
HTM	10	1·7±0·1	1·7±0·2	1·9±0·3
Thyroid 27-hr RAIU (%)				
HTG	10	8·1±1·7	7·7±3·2	8·1±1·1
RTM	9	8·5±1·2	4·5±0·7*	10·9±0·8
HTM	10	7·2±1·1	6·7±0·9	12·3±1·0*

* Differs significantly from pre-immunization value ($P<0·05$ or lower by paired t-test).

DISCUSSION

Immunization of rabbits with the microsomal fraction of rabbit or human thyroid tissue resulted in the production of thyroid-stimulating material (RITS) by some of the animals. The nature of the antigen which causes this apparently immunological response is unknown. The response to rabbit tissue indicates that the antigen is not species specific. The lack of development of RITS in response to immunization with HTg confirms our hypothesis that HTg is not the antigen involved in the generation of this thyroid stimulating γ-globulin. Whatever else may be in the microsomal fraction, its small contamination with HTg cannot be the responsible entity.

The frequency of RITS response to immunization was much less in this group of animals than in our previous studies. Previously, immunization with HTM was followed in about two-thirds of the animals by the production of significant amounts of RITS. The animals used this time were obtained from a different supplier than the Dutch rabbits that we used in the past. Also, to simplify their care, they were not immunized with footpad injections as had been done previously. It is possible that either or both of these changes resulted in a lower incidence of RITS.

Animals with RITS activity have not appeared to have thyroid hyperfunction. It seems likely that thyroid hyperfunction does not develop because the thyroid-stimulating substance is prevented from exerting its effect on the thyroid by the presence of other, perhaps inhibitory, antibodies in the serum. We felt that immunization with rabbit tissue might tend to produce fewer interfering antibodies and therefore a situation more akin to human Graves' disease than occurs following immunization of rabbits with human tissue. We also wanted to use allogeneic antigen because of the experiences others have had with experimental thyroditis which suggested that allogeneic tissue might produce an experimental immunological disease under conditions in which xenogeneic tissue would not.

Experimental thyroiditis in rabbits has been produced by immunization with rabbit thyroglobulin in Freund's adjuvant while human thyroglobulin in Freund's adjuvant has not produced thyroiditis (Terplan *et al.*, 1960). Large amounts of soluble human thyroglobulin without Freund's adjuvant will produce experimental thyroiditis in rabbits (Weigle & Nakamura, 1967). In either case, the disease seems to be a result of a break in tolerance to the native protein (Weigle, High & Nakamura, 1969).

If an experimental model of Graves' disease could be produced by similar mechanisms, then RTM might be superior to HTM as an antigen. The results, however, contain no conclusive support for this idea. The incidence of RITS was similar in the RTM and HTM immunized groups. The serum-free T_4 concentration of the RTM group did increase slightly, but this was not reflected in any consistent increase in thyroid radio-iodine uptake nor were the amounts of thyroid-stimulating activity found in the serum of these animals larger than in the HTM-immunized group.

Immunization with HTg produced high titres of thyroglobulin haemagglutinins, colloid-binding antibodies by immunofluorescence and thyroiditis. The incidence and severity of thyroiditis in rabbits immunized with soluble human thyroglobulin in this study was similar to that described by Weigle & Nakamura (1967). Since human thyroglobulin in Freund's adjuvant does not produce thyroiditis in rabbits (Terplan *et al.*, 1960), our failure to do so with the small amounts* of thyroglobulin which may have contaminated the microsomal fraction was expected.

This study confirms previous reports that the response of the animals to thyroglobulin in terms of anti-thyroglobulin antibodies does not correlate well with the occurrence of thyroiditis (Rose *et al.*, 1965). This lack of correlation is not surprising if one considers the variability in antigenic determinants, antibody responses and hypersensitivity phenomena that may exist.

What is the nature of the thyroid-stimulating material, RITS? It is tempting to believe that this material is an experimental counterpart to the long-acting thyroid stimulator (LATS). of Graves' disease This notion is supported by our studies, mentioned earlier, showing RITS activity in γ-globulin fractions of serum, neutralization by anti-rabbit γ-globulin and production despite the administration of large amounts of T_4. The current studies add still further evidence against the possibility that TSH is somehow responsible for RITS activity. If RITS were simply TSH, or TSH bound to globulin one might expect it to correlate with the presence of thyroiditis and consequent hypothyroidism, but thyroiditis was not present in RITS-producing animals.

Anti-thyroglobulin antibodies and binding of T_4 by γ-globulin have been demonstrated in nearly all the animals in this study immunized by various means. Such binding of T_4 can produce McKenzie assay results which suggest thyroid stimulation. We have recently described, however, a double-isotope modification of the McKenzie assay which makes it possible to distinguish thyroidal from extra-thyroidal effects of materials injected into the assay mice (Florsheim *et al.*, 1970). This procedure has clearly indicated that sera containing RITS promote the release of ^{131}I tagged materials from the thyroid gland as well as slowing the net clearance of $[^{125}I]T_4$ from the serum. Thus RITS clearly stimulates thyroid hormone secretion. Whether it stimulates other aspects of thyroid function is currently being studied.

The following phenomena are known to arise following immunization of rabbits with various thyroid fractions: thyroiditis, production of anti-thyroglobulin and anti-cytoplasmic

* The total protein content of these fractions was less than 1 mg/g-eq.

antibodies, elevation in serum total T_4 (which is clearly related to a γ-globulin T_4-binder in these sera), RITS activity and a T_4-binding activity which slows the disappearance from serum of [^{125}I]T_4. The relationships of these various phenomena to each other have only been partially exposed by the present study; they are currently the subject of further investigations.

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REFERENCES

BEALL, G.N., DANIEL, P.M., PRATT, O.E. & SOLOMON, D.H. (1969a) Effects of immunization of baboons with human thyroid tissue. *J. clin. Endocr.* **29,** 1460.

BEALL, G.N., DONIACH, D., ROITT, I. & EL KABIR, D. (1969b) Inhibition of the long-acting thyroid stimulator (LATS) by soluble thyroid fractions. *J. Lab. clin. Med.* **73,** 988.

BEALL, G.N. & SOLOMON, D.H. (1966) Inhibition of long-acting thyroid stimulator by thyroid particulate fractions. *J. clin. Invest.* **45,** 552.

BEALL, G.N. & SOLOMON, D.H. (1968) Thyroid-stimulating activity in the serum of rabbits immunized with thyroid microsomes. *J. clin. Endocr.* **28,** 503.

DERRIEN, Y., MICHEL, R. & ROCHE, J. (1946) Recherche sur la preparation et les proprietes de la thyroglobuline pure. *Biochim. biophys. Acta (Amst.),* **2,** 454.

FLORSHEIM, W.H., SOLOMON, D.H., CHOPRA, I.J. & BEALL, G.N. (1970) Thyroid and extrathyroid responses to serum from thyroid-immunized rabbits. *Clin. Res.* **18,** 168.

FULTHORPE, A.J., ROITT, I.M., DONIACH, D. & COUCHMAN, K.G. (1961) A stable sheep cell preparation for detecting thyroglobulin autoantibodies and its clinical applications. *J. clin. Path.* **14,** 654.

MCKENZIE, J.M. (1968) Experimental production of a thyroid-stimulating antithyroid antibody. *J. clin. Endocr.* **28,** 596.

MURPHY, B.E.P. (1965) The determination of thyroxine by competitive protein-binding analysis employing an anion-exchange resin and radiothyroxine. *J. Lab. clin. Med.* **66,** 161.

MURPHY, B.E.P. & PATTEE, C.J. (1964) Determination of thyroxine utilizing the property of protein-binding. *J. clin. Endocr.* **24,** 187.

PINCHERA, A., LIBERTI, P. & BADALAMENTI, G. (1966) Long acting thyroid stimulator and thyroid antibodies. *Lancet,* **i,** 374.

PREMACHANDRA, B.N., RAY, A.K., HIRATA, Y. & BLUMENTHAL, H.T. (1963) Electrophoretic studies of thyroxine and triiodothyronine binding by the sera of guinea pigs immunized against thyroglobulin. *Endocrinology,* **73,** 735.

ROSE, N.R., KITE, J.H., DOEBBLER, T.K., SPIER, R., SKELTON, F.R. & WITEBSKY, E. (1965) Studies on experimental thyroiditis. *Ann. N.Y. Acad. Sci.* **124,** 201.

SCHUSSLER, G.C. & PLAGER, J.E. (1967) Effect of preliminary purification of ^{131}I-thyroxine on the determination of free thyroxine in serum. *J. clin. Endocr.* **27,** 242.

SOLOMON, D.H. & BEALL, G.N. (1968) Thyroid-stimulating activity in the serum of immunized rabbits. II. Nature of the thyroid-stimulating material. *J. clin. Endocr.* **28,** 1496.

SOLOMON, D.H. & BEALL, G.N. (1970) The effect of thyroxine on the thyroid stimulating activity in the serum of rabbits immunized with thyroid tissue. *Endocrinology,* **86,** 191.

STERLING, K. & BRENNER, M.A. (1966) Free thyroxine in human serum: Simplified measurement with the aid of magnesium precipitation. *J. clin. Invest.* **45,** 153.

TERPLAN, K.L., WITEBSKY, E., ROSE, N.R., PAINE, J.R. & EGAN, R.W. (1960) Experimental thyroiditis in rabbits, guinea pigs and dogs, following immunization with thyroid extracts of their own and of heterologous species. *Amer. J. Path.* **36,** 213.

WEIGLE, W.O. (1965) The induction of autoimmunity in rabbits following injection of heterologous or altered homologous thyroglobulin. *J. exp. Med.* **121,** 289.

WEIGLE, W.O., HIGH, G.J. & NAKAMURA, R.M. (1969) The role of mycobacteria and the effect of proteolytic degradation of thyroglobulin on the production of autoimmune thyroiditis. *J. exp. Med.* **130,** 243.

WEIGLE, W.O. & NAKAMURA, R.M. (1967) The development of autoimmune thyroiditis in rabbits following injection of aqueous preparations of heterologous thyroglobulins. *J. Immunol.* **99,** 223.

ABBREVIATIONS

HTg human thyroglobutin
HTM human thyroid microsomal fraction
RAIU radioiodine uptake
RITS rabbit immunologic thyroid stimulator
RTM rabbit thyroid microsomal fraction