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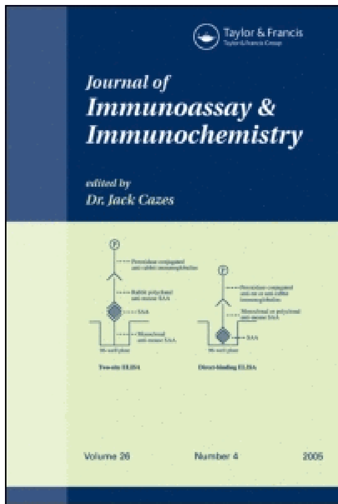

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Validation of the Cytochemical Section Bioassay for Thyroid Stimulating Antibodies

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VALIDATION OF THE CYTOCHEMICAL SECTION BIOASSAY FOR THYROID STIMULATING ANTIBODIES

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ABSTRACT

A cytochemical bioassay for thyroid stimulators which utilised 12 um sections of guinea pig thyroid as the target tissue was validated and applied to the measurement of thyroid stimulating antibodies (T.S.Ab.) in human plasma. T.S.H. and T.S.Ab. could be distinguished in the assay by the times at which they produced maximal responses; 90 seconds for T.S.H. and 180 seconds for T.S.Ab. Homogeneity of response to stimulation was relatively uniform throughout thyroid tissue enabling simultaneous measurement of several plasma samples per guinea pig. Sensitivity of the assay was 1.5×10^{-9} mU/l MRC L.A.T.S.-B. Antiserum to H-T.S.H. produced no significant change in T.S.Ab.-like activity when added to plasma from a thyrotoxic patient. Both anti-IgG and aliquots of a homogenate of thyroid tissue from a thyrotoxic patient diminished responses of thyrotoxic plasma in a concentration dependent manner. T.S.Ab. was present at titres of 1/100 to 1/100,00 in 13 hyperthyroid patients tested to date.

Since the discovery by Adams and Purves (1) that serum from a thyrotoxic patient exerted a prolonged effect in the bioassay of T.S.H. many assays have been developed to measure this entity. These assays may be divided into two main categories; those which

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measure a stimulatory effect on the thyroid gland and others which depend on binding to a component of human thyroid tissue.

The best known of the "stimulatory" assays, the mouse bioassay of the long acting thyroid stimulators (LATS) (2), yielded a relatively low number of positive results although concentrates of such sera increased the frequency of detection (3). The description of a LATS protector (LATS-P) (4) resulted in a greatly increased frequency of detection of abnormal thyroid stimulators in the sera of hyperthyroid patients (5, 6). Other "stimulatory" assays for thyroid stimulating antibodies (T.S.Ab.) have been developed using human thyroid tissue as the target organ and measuring entities such as colloid droplet formation, adenyl cyclase, activity, or cyclic AMP formation (7-10). These assays resulted in increased detection of T.S.Ab. compared to the McKenzie assay.

The second category of assays for T.S.Ab. are the radioreceptor or displacement assays (11,12). These assays depend on the displacement of ^{125}I labelled T.S.H. from human thyroid membranes by T.S.Ab. Using such assays T.S.Ab. has been detected in almost all sera from thyrotoxic patients tested (12-13).

The application of cytochemical bioassay (C.B.A.) to the measurement of thyroid stimulators in human plasma (15) provided a "stimulator" assay which could detect both T.S.H. and LATS with much greater sensitivity than currently available bioassays. The C.B.A. as originally described measured the effects of thyroid stimulators on lysosomal membrane permeability in guinea pig thyroid segments. This method has restrict-

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ed application in that it permitted the estimation of only one plasma sample per animal. A preliminary communication on the development of a cytochemical bioassay for thyroid stimulators which utilized 12 um sections rather than segments of thyroid tissue and therefore permitted the simultaneous assay of larger numbers of specimens per assay was reported by Gilbert et al (16). The present report describes the validation and application of such a section assay with particular reference to the measurement of T.S.Ab. in human plasma.

MATERIALS AND METHODS

A comparison of the procedures used in the segment ana section cytochemical bioassays is shown in Table 1. The methodology is identical up to the third step. In the section assay it is sections

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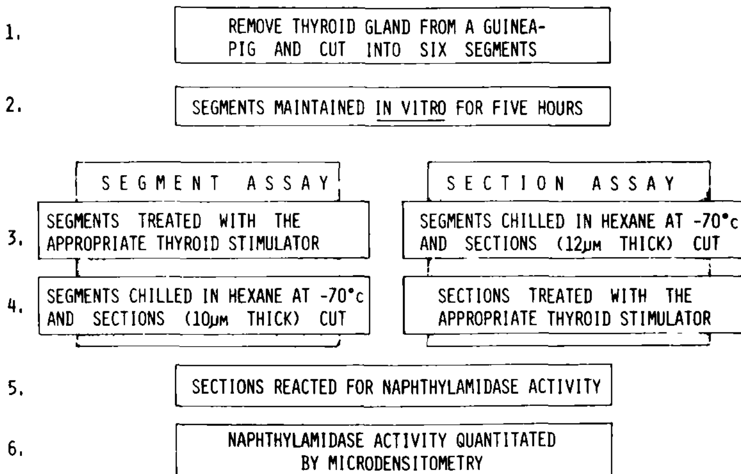
TABLE 1
PROCEDURES FOR THE SEGMENT AND SECTION BIOASSAYS



rather than segments of thyroid tissue that are treated with the thyroid stimulator.

Culture and Freezing

In the section assay each lobe of a thyroid gland removed from a female guinea pig (Hartley strain; weight $<$ 300g) was bisected and maintained in vitro for 5 hrs. in Trowells T-8 medium in an incubator at 37°C (17). After 5 hrs. each of the segments was chilled to - 70°C in a beaker containing n-hexane suspended in an ice bath prepared by adding crushed solid CO_2 to methanol. The tissue was stored at this temperature in a thermos flask containing solid CO_2 and used within 2 days.

Preparation of Sections:

Sections (12 um thick) were cut in a cryostat with motorized cutting action (Slee Model HRM) with cabinet temperature at -30°C in which the knife was cooled with solid CO_2. The sections were picked up from the knife onto glass slides and stored in the cryostat chamber until ready for reaction. Slides with sections mounted were allowed to dry at room temperature immediately before use and were then clipped back to back in duplicate into the lid of a box made up of 24 chambers so that each pair of slides fitted into one chamber. This apparatus was similar to that developed for the ACTH cytochemical section bioassay (18). The slides were allowed to warm up to 37°C for not less than 5 minutes before they were exposed to the stimulator.

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Cytochemical Methods:

The chambers of the section apparatus were filled with 4 ml. of the appropriate dilution of either T.S.H., LATS-B or a plasma sample. The thyroid stimulators were diluted in a "carrier medium" at 37°C consisting of 0.1% gum tragacanth and 0.05 M sodium acetate in T-8 medium at pH 7.6 (16). This pH was achieved by adding 1.6 ml of IM HCL per 100 ml of carrier medium and mixing thoroughly to remove the bicarbonate as CO_2. The pH was checked and adjusted, if necessary, just prior to use.

The sections were next exposed to either standards or plasma diluted in carrier medium. After exposure for the appropriate period the sections were reacted for naphthylamidase activity as previously described (15). After 6 minutes the reaction was stopped by immersing the sections in 0.85% sodium chloride. The slides were unclipped and placed in Coplin jars containing 0.1M copper sulphate for not less than 2 minutes and the sections were mounted in Farrant's medium pH 6.5. These cytochemical methods have been previously described (19). The slides were stored in the dark for up to one week before the reaction product was measured at 550 nm using an M-85 scanning and integrating microdensitometer (Vickers Instruments). Readings obtained by microdensitometry can be expressed directly as relative absorption which is an arbitrary machine unit. However, to permit comparison between readings obtained at different times or on different instruments results may be quantitated in absolute terms as integrated extinction.

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Exposure of sections to the thyroid stimulators and the subsequent naphthylamidase reaction were carried out in a specially constructed incubator permitting easy handling of the slide holder and reaction chambers while maintaining a constant temperature.

Subjects:

Plasma samples were obtained from 13 patients who were both clinically and biochemically thyrotoxic. None of the patients had undergone previous therapy for thyroid disease. Eight were females and five males. Ages ranged from six months to sixty-five years. Five non-hospitalised euthyroid volunteers, three females and two males, served as controls. Blood samples were collected into lithium-heparin tubes. The plasma was separated immediately following venipuncture, snap frozen at -70°C in methanol containing solid CO_2, and stored at -70°C in a freezer.

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Statistical Methods:

Statistical analysis was performed using Student's 't' test.

RESULTS

In order to establish homogeneity of response of sections of thyroid tissue in terms of naphthylamidase activity the following studies were carried out on sections which had been stimulated with 10^{-2} mU/l T.S.H. Naphthylamidase activity was measured as described under "Methods" and results are expressed as relative absorption.

Intra-follicle variation:

The naphthylamidase activity of every cell from each of 4 follicles in one section was measured. The

coefficients of variation were 7.0%,8.9%.9.7% and 5.9% respectively (Mean 7.9%).

Intra-section variation:

The intra-section variation was achieved by measuring one cell from each intact follicle (n=24) within a section. The coefficient of variation was 9.2%.

Intra and Inter-segment variation:

A total of 24 sections were cut, six from each of 4 segments of a guinea pig thyroid gland. Mean values ± S.D. and intra-segment coefficients of variation in sections cut from the 3 segments are shown in Table II. The mean inter-segment coefficient of variation was 8.35%

These results showed that the response throughout stimulated thyroid tissue was relatively homogeneous, and when necessary, sections could be used from any of the four segments from a guinea pig thyroid gland. Routinely two 12 um sections were cut for each standard or dilution of plasma assayed. Measurements of individual sections were carried out on one cell from each of 10 follicles. Thus, readings from a total of twenty cells were utilised for each standard point or dilution of plasma assayed. To prevent operator bias in performing microdensitometric readings a "blind" assay design was always used.

Influence of Time on Response of Thyroid Sections to T.S.H. and LATS:

T.S.H. and thyroid stimulating antibodies can be distinguished in this assay by the different times at which they cause maximal labilization of thyroid lysosomal membranes (15,16). Sections of thyroid gland were exposed to either T.S.H.(10^{-2}mU/l) or to MRC LATS-B

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TABLE 2

INTRA AND INTER-SEGMENT VARIATIONS
(RELATIVE ABSORPTION MEAN ± S.D.)

SECTION	SEGMENT			
	1	2	3	4
A	177 ± 14.5	181 ± 16.8	174 ± 15.3	180 ± 13.1
B	177 ± 8.0	178 ± 13.7	176 ± 11.0	176 ± 10.1
C	171 ± 19.0	171 ± 10.7	182 ± 17.9	174 ± 12.1
D	174 ± 19.8	177 ± 11.6	167 ± 13.9	177 ± 15.2
E	168 ± 17.1	169 ± 17.8	174 ± 15.1	174 ± 15.0
F	170 ± 6.2	170 ± 13.5	173 ± 13.3	174 ± 19.5
MEAN ± S.D.	173.5± 14.5	174.0± 14.6	174.3± 14.6	175.8± 14.1
C.V. %	8.6	8.4	8.4	8.0

(1.5×10^{-6}mU/l) for varying times. It can be seen from Fig.1 that there was a single peak of T.S.H. activity occurring at 90 seconds. In contrast to the effects observed for T.S.H. the maximal response to LATS-B occurred at 180-210 seconds. The effect of the carrier medium was minimal up to 120 seconds but after this it was beginning to exert an influence on the lysosomal membranes as shown by the gradual increase in naphthylamidase activity from 120 to 210 seconds.

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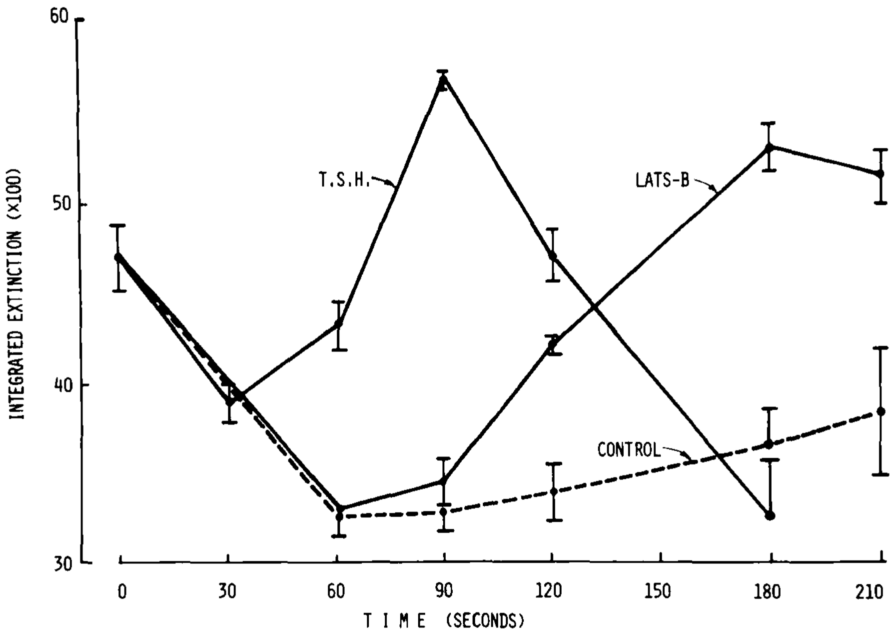

Figure 1. Influence on naphthylamidase activity of time of exposure to T.S.H. (10^{-2} mU/l) and M.R.C. L.A.T.S.-B (1.5×10^{-6}mU/l). The horizontal bars represent the mean values of ten readings from duplicate slides. The solid dots represent the average value of the duplicates.

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LATS-B Standard Curve:

The response of thyroid sections to graded concentrations of M.R.C. LATS-B standards are shown in Fig.2. The sections were exposed to the standard preparation for 180 seconds. The lower limit of detection of this preparation was 1.5×10^{-9} mU/l. The standard curve was linear from 1.5×10^{-8} mU/l to 1.5×10^{-5} mU/l but as shown in Fig.2 an apparent decrease in activity was observed at higher concentrations. A plasma pool obtained from 4 euthyroid volunteers did not show any detectable stimulatory activity at the dilutions tested, nor did the 'carrier medium' alone.

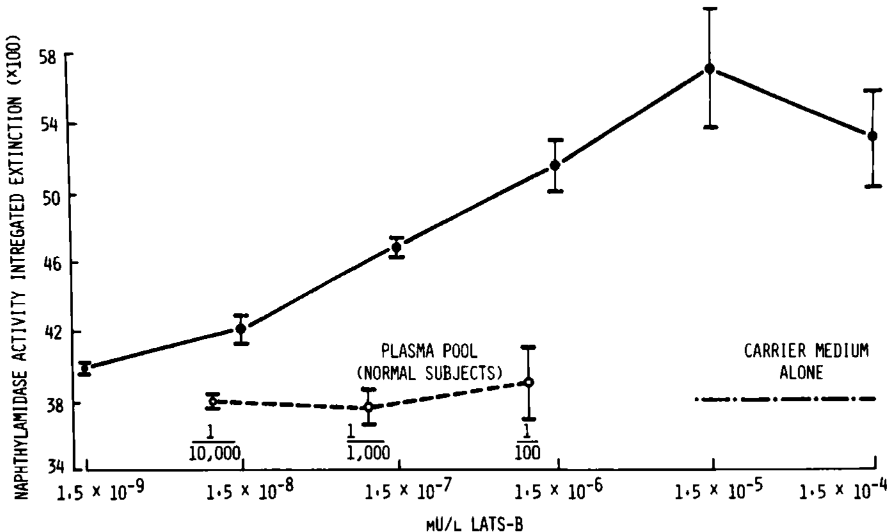

Figure 2. L.A.T.S.-B standard curve. The horizontal bars represent the mean values of ten readings from duplicate slides. The solid dots represent the average value of the duplicates.

Parallelism of responses in human plasma:

Naphthylamidase activities expressed as integrated extinction obtained following stimulation of thyroid sections by various dilutions of plasma from thyrotoxic patients are shown in Fig.3. As with the standard preparation of LATS, higher concentrations of T.S.Ab. (i.e.lower dilutions of plasma) sometimes resulted in an apparent decrease in stimulatory activity. In one patient in whom a range of dilutions of plasma from 10^{-2} to 10^{-6} were tested the biphasic nature of response to T.S.Ab. is clearly demonstrated. Apparent maximal stimulation occurred with a dilution of 10^{-4} of this sample and higher concentrations of plasma resulted in a dec-

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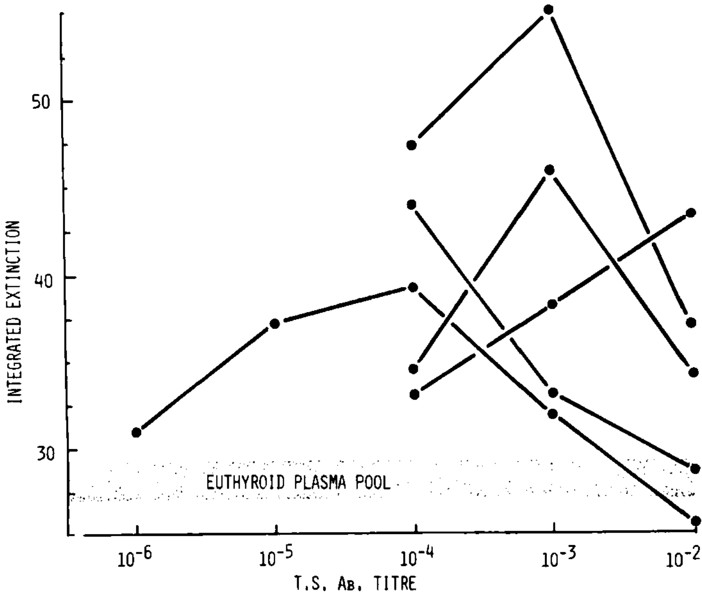

Figure 3. Assay responses of plasma dilutions from individual thyrotoxic patients.

rease in integrated extinction which at a dilution of 10^{-2} fell below that of the euthyroid plasma pool.

Specificity:

The specificity of the assay for thyroid stimulating antibodies was investigated by adding to a 1/1,000 dilution of plasma from a thyrotoxic patient varying dilutions of anti-H-T.S.H., anti-human IgG or human thyroid homogenate. The human thyroid homogenate was prepared from the thyroid gland of a patient who had undergone surgery for hyperthyroidism (20).

(1) Antiserum to H-T.S.H.at 1/10,000 and 1/500,000 dilutions did not significantly alter the stimulatory response of the thyrotoxic plasma observed at 180 seconds. The T.S.H. antiserum alone did not possess significant stimulatory activity over the control euthyroid plasma pool at either dilution of antiserum used.

(2) The effects of anti-human IgG or human thyroid homogenate are shown in Fig.4a. Anti-human IgG at 1/1,000 and 1/100 dilutions almost abolished the stimulatory effects of the thyrotoxic plasma. A reduction in stimulatory activity was also observed at a 1/10,000 dilution of anti-human IgG but this was not significant. Anti IgG alone did not produce significant stimulatory activity. The human thyroid homogenate diminished the stimulatory effect of the thyrotoxic plasma at all concentrations of homogenate used as shown in Fig.4b. However, the homogenate itself had a stimulatory effect on the thyroid sections which was significantly greater than that produced by the euthyroid plasma pool at the highest concentration of homogenate used (50 ul/ml) ($P < 0.005$).

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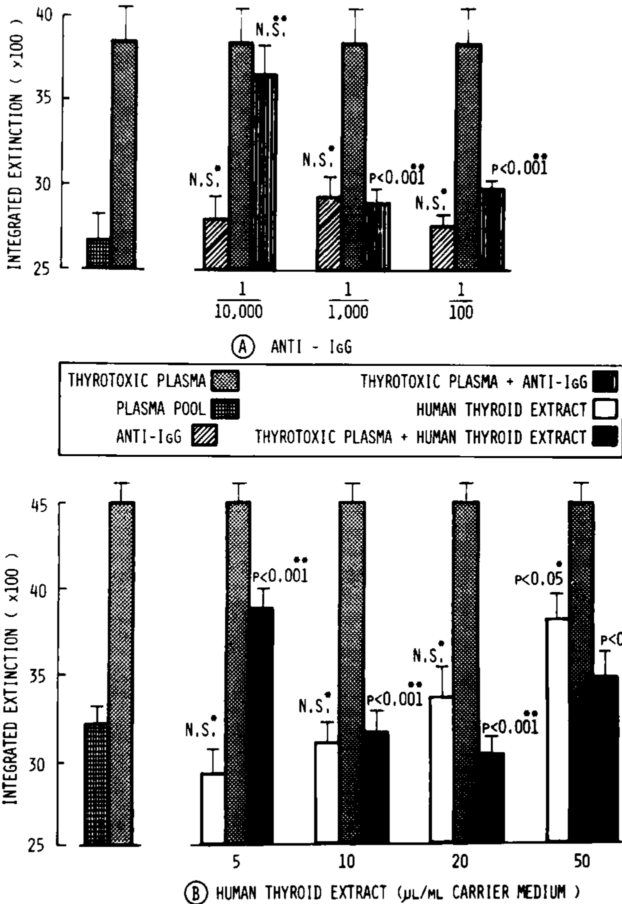

Figure 4. Effect of (a) anti-human IgG and (b) aliquots of a homogenate of thyroid tissue from a thyrotoxic patient on stimulatory activity in plasma from thyrotoxic patients. Variation between duplicate sections is shown by the horizontal bars. Statistical analysis refers to differences from control plasma pool* or from the thyrotoxic plasma alone** (N.S.= not significant).

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Measurement of T.S.Ab. in Human Plasma:

T.S.Ab. measurements on an initial group of 13 patients who had Graves' disease are shown in Fig.5. Results are expressed as the maximum dilution at which a response significantly elevated with respect to the control plasma pool ($P < 0.05$) was obtained in the assay. Titres varied from 1/100 to 1/100,000. Plasma samples from 5 euthyroid volunteers showed no stimulatory activity at 1/100, 1/1,000 or 1/10,000 dilutions and the pooled plasma from these subjects was consequently used in the assays as a control.

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Collection of Plasma Specimens:

Parallelism of response to T.S.Ab. in the section assay may be dependent on the method of sample collection. Responses to plasma from a thyrotoxic patient were found to be parallel to a standard preparation when the plasma was separated from red cells and chilled to -70°C immediately after venipuncture. Similar findings were observed if the plasma was separated immediately and allowed to stand at room temperature for 2½ hours. However, non-parallel responses occurred if the blood sample was

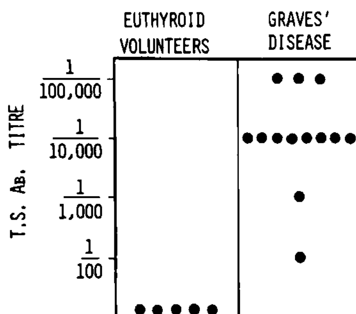

Figure 5. T.S.Ab. titres in euthyroid volunteers and in patients who had Graves' disease.

allowed to stand at room temperature for 2½ hours before separation. The reproducibility of this finding has not been established and pending such studies it seemed preferable that the blood samples be separated as soon as possible after venipuncture. All samples investigated in the present study were treated in this way.

DISCUSSION

These studies have shown that the response to stimulation in terms of intralysosomal naphthylamidase activity was relatively uniform throughout guinea pig thyroid tissue. The findings of coefficients of variation for such activity of less than 10% both within and between segments of thyroid tissue permits the utilization of sections from different segments of tissue within a single assay. This finding greatly increases the scope of cytochemical bioassay in that it allows simultaneous measurement of much larger numbers of specimens than was possible using the earlier segment assay. The segment assay for T.S.H. as described by Bitensky et al (15) permitted the estimation per guinea pig of only one plasma at two dilutions together with four standards. Similarly two plasmas could be assayed for T.S.Ab. The section assay described above can comfortably include in one T.S.Ab. assay five plasmas at three dilutions or seven plasmas at two dilutions. Large numbers of samples could be processed in a single assay but operator fatigue in carrying out microdensitometric readings represents a limiting factor.

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Previously reported cytochemical bioassays for T.S.H.(15,15,21)yielded a sensitivity considerably greater than other bioassays for this hormone and in fact at 10^{-4} mU/l the sensitivity was approximately 10^{4} times greater than currently available T.S.H.

radioimmunoassays. The sensitivity of 1.5×10^{-9} mU/l for M.R.C. L.A.T.S.-B achieved in the present study is also dramatically more sensitive than comparable "stimulatory" assays for T.S.Ab. Examples of other bioassays for T.S.Ab. with sensitivities reported in terms of L.A.T.S.-B were a modification of the McKenzie assay (22) which yielded a sensitivity of 6.25 mU/animal while Shishiba et al,(23) measuring colloid droplet formation reported a sensitivity for L.A.T.S. of 0.24 mU/animal.

Although the sensitivity of cytochemical section bioassay for T.S.Ab. is exquisitively low the working range of 1.5×10^{-8} mU/l to 1.5×10^{-5} mU/l is somewhat narrow. The reason for this is that the lysosomal membranes were probably maximally labilised at 1.5×10^{-5} mU/l and that further increasing the concentration of L.A.T.S. resulted in supra-optimal labilisation with leakage of enzyme from the lysosomes and a fall in integrated extinction (15). Such supra-optimal labilisation probably accounts for the lack of parallelism shown by some of the plasma from thyrotoxic patients investigated in the present study and emphasises the necessity for assaying T.S.Ab. at a number of dilutions in order to establish a positive dose-response relationship and thus prevent false negative results. This finding is in keeping with the report of Loveridge et al (24) who demonstrated biphasic responses to T.S.Ab. in human plasma using a cytochemical segment bioassay.

The specificity for T.S.Ab. of the cytochemical section bioassay has been established using antisera to H-T.S.H., anti-human IgG and an extract of human thyroid tissue from a thyrotoxic patient. From these results it is apparent that the stimulatory activity observed at 180 secs. is independent of T.S.H. and resides in a substance immunologically similar to IgG.

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The neutralisation of T.S.Ab. stimulatory activity by homogenate of human thyroid tissue offers further evidence that the activity being measured binds to a component of human thyroid tissue. However, the demonstration that the human thyroid homogenate itself possessed stimulatory activity was a surprising finding. The origin of such stimulatory activity is currently under investigation but it may represent endogenous T.S.Ab. bound to the thyroid tissue or some cellular material released during homogenisation which caused labilisation of lysosomal membranes.

The finding of positive titres of T.S.Ab. in all hyperthyroid patients investigated to date in the section assay is an encouraging finding. The exquisite sensitivity of the technique, its specificity, together with the new found possibility of simultaneously assaying larger numbers of specimens should provide a useful contribution to the investigation of hyperthyroidism.

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REFERENCES

1. Adams,D.D.and Purves,H.D. Abnormal responses in the assay of thyrotrophin. Proceedings University of Otago Medical School, New Zealand,1956;34:11-12.

2. McKenzie, J.M. The bioassay of thyrotrophin in serum. Endocrinology (1958)63, 372-382.

3. Carneiro,L., Dorrington,K.J. and Munro,D.S. Recovery of the long-acting thyroid stimulator from serum of patients with thyrotoxicosis by concentration of immunoglobulin. Clin.Science (1966); 31, 215-221.

4. Adams,D.D. and Kennedy, T.H. Occurrence in thyrotoxicosis of a gamma globulin which protects LATS from neutralization by an extract of thyroid gland. J.Clin Endocr Metab (1967); 27, 173-177.

5. Adams,D.D.,Kennedy,T.H.,Stewart,R.D.H. Correlation between long-acting thyroid stimulator protector level and thyroid I-131 uptake in thyrotoxicosis. British Medical Journal (1974); 2: 199-201.

6. Dirmikis,S., Kendall-Taylor,P. and Munro,D.S. The nature and significance of LATS-protector. In Eds. J.Robbins and J.E.Braverman. Thyroid Research, Excerpta Medica, Amsterdam (1976); 403-406.

7. Onaya,T., Kotani,M., Yamada,T. and Ochi,Y. New in vitro tests to detect the thyroid stimulator in sera from hyperthyroid patients by measuring colloid droplet formation and cyclic AMP in human thyroid slices. J.Clin Endocr Metab (1973); 36: 859-866

8. Orgiazzi,J., Williams,D.E.,Chopra,I.J. and Solomon, D.H. Human thyroid adenyl cyclase-stimulating activity in immunoglobulin G of patients with Graves' disease. J.Clin Endocr Metab (1976); 42: 341-354.

9. McKenzie,J.M. and Zakarija,M. LATS in Graves' disease. Recent Progr Hormone Res (1977); 33: 29-53.

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10. Shishiba,Y.,Miyachi,Y.,Takaishi,M.and Ozana,Y. LATS-protector activity in thyrotoxicosis measured by thyroidal intracellular colloid droplet formation. J.Clin Endocr Metab (1978); 45: 841-848.

11. Smith,B.R.and Hall,R. Thyroid-stimulating immunoglobulins in Graves' disease. Lancet (1974); ii: 427-430.

12. O'Donnell,J.,Troukoudes,K.,Silverberg,J., Row,V. and Volpe,R. Thyrotrophin displacement activity of serum immunoglobulins from patients with Graves' disease. J.Clin Endocr Metab (1978); 45: 770-777.

13. Mukhtar, E.D. Smith,B.R., Pyle,G.A.,Hall,R and Vice,P. Relation of thyroid-stimulating immunoglobulin to thyroid function and effects of surgery, radioiodine and antithyroid drugs. Lancet (1975); i: 904-906.

14. Brown,R.S., Jackson,I.M.D.,Pohl,S.L. and Reichlin,S. Do thyroid-stimulating immunoglobulins cause non-toxic and toxic multinodular goitre ? Lancet (1978); i: 904-906.

15. Bitensky,L., Alaghband-Zadeh,J. and Chayen,J. Studies on thyroid stimulating hormone and the long-acting thyroid stimulating hormone. Clin Endocr (1974); 3: 363-374.

16. Gilbert,D.M.,Besser,G.M.,Bitensky,L. and Chayen,J. Development of a cytochemical section-assay for thyroid stimulators. J.Endocr.(1977); 75: 40p.

17. Trowell,O.A. The culture of mature organs in a synthetic medium. Exp Cell Research (1959); 16: 118-147.

18. Alaghband-Zadeh,J., Daly,J.R. Bitensky,L., Chayen,J. The cytochemical section bioassay of corticotrophin. Clin Endocr (1974): 3: 319-327.

19. Chayen,J., Bitensky,L. and Butcher,R. Practical Histochemistry (1973). John Wiley & Son Ltd. New York, London.

20. Adams, D.D.,Kennedy,T.D. Evidence to suggest that LATS-protector stimulates the human thyroid gland. J.Clin Endocr Metab (1971): 33: 47-51.

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21. Petersen.V.,Smith,B.R.and Hall,R. A study of thyroid stimulating activity in human serum with the highly sensitive cytochemical bioassay. J.Clin Endocr Metab (1975); 41: 199-202.

22. Mason,E.K.,Hetzel,B.S.,Good,B.F.and Stenhouse, N.S. An improved bio-assay for the determination of long-acting thyroid stimulator (LATS). J Clin Endocr Metab (1967); 27: 1529-1539.

23. Shishiba,Y.,Miyachi,Y.,Takaishi,M. and Ozana,Y. The relation between LATS-protector and human thyroid stimulator. Proceedings of V International Congress of Endocrinology, Excerpta Medica, Amsterdam-Oxford (1977); 2: 467-473

24. Loveridge,N., Zakarija,M.,Bitensky,L.,and McKenzie,J.M. The cytochemical bioassay for thyroid stimulating antibody of Graves' disease: further experience. J Clin Endocr Metab (1979); 49: 610-615.

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