THE EFFECTS OF THYROTROPIN AND THE LONG ACTING THYROID STIMULATOR ON ADIPOSE TISSUE

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RUNNING TITLE

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ABSTRACT

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Thyrotropin (TSH) and the long-acting thyroid stimulator (LATS) have similar actions on glucose metabolism in adipose tissue in vitro. Both thyroid stimulators cause enhancement of lipolysis when added to adipose tissue slices or free fat cells from guinea pig, rat and human but LATS has no effect on that from mice. LATS is not as rapidly bound by adipose tissue and has a delayed onset of action compared to TSH. Both mediate lipolysis via adenyl cyclase.

The lipolytic effect is a more sensitive assay for the detection of LATS than the mouse bioassay and using this technique LATS is detectable in the serum of 91% of Graves' disease patients.

RESUME

EFFETS DE LA THYROTROPINE ET DU LONG-ACTING THYROID STIMULATOR SUR LA TISSU ADIPEUX

PAR

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La thyrotropine et le "long-acting thyroid stimulator" agissent de façon semblabe sur le métabolisme du glucose dans le tissu adipeux in vitro. Ces deux stimulants de la thyroide provequent une augmentation de la lipolyse dans les tranches de tissu adipeux ou dans le cellules graisseuses isoleés du cochon d'Inde, du rat et de l'homme, toutefois LATS ne produit pas cet effet chez la souris.

LATS ne s'attache pas aussis rapidement au tissu adipeux et son action initiale est retardés en couparaison de celle de TSH. Toutes deux agissent sur la lipolyse par l'intermédiaire de l'adényl cyclase.

L'effet lipolytique est un essai plus sensitif pour détecter LATS que l'essai biologique sur la souris. Cette technique permet de détecter LATS dans le sérum de 91% de patients affligés de la maladie de Basedow.

CONTENTS

This thesis is submitted in the form of five original papers suitable for submission to scientific journals for publication as is currently (1973) allowed under thesis regulation section 4.2.7.(h). All of the work presented was performed whilst I was enrolled in the M.Sc. course in Experimental Medicine at McGill University between 1967 and 1972.

The five papers are joined together by brief notes in an attempt to attain overall cohesiveness. For the same reason an historical overview of the topic is given in the introduction. Each paper has its own bibliography in the format prescribed by the journal to which we intend to submit the paper or in which it was published. The introduction and summary-conclusions have their own independently numbered bibliography at the end of the thesis.

INTRODUCTION

The subject matter of this thesis falls naturally into the following topics -

(1) Thyrotropin (TSH)

(2) The Long Acting Thyroid Stimulator and the Pathogenesis of Graves' disease

(3) Actions of Thyrotropin and the Long Acting Thyroid Stimulator

(4) Hormonal Effects on Adipose Tissue

- and these will be discussed separately in the introduction.

1. THYROTROPIN (TSH)

That there is some relationship between the pituitary and the thyroid gland has been known since Niepce (1) described enlargement of the anterior pituitary in patients with goitre or cretinism (1851) and Rogowitsch (2) reported increased cellularity of the adenohypophysis after thyroidectomy in animals in 1888. Viguier (3) reported an increased number of acidophils (although all future work has shown the thyrotroph to be a basophil) in the pituitary of the lizard U. Acanthinurus in 1911. Smith (4) and Allen (5) demonstrated in 1916 that, like thyroidectomy, removal of the pituitary

anlage blocked tadpole metamorphosis. Gudernatsch (6) in 1912 had shown that thyroid extract given to thyroidectomized tadpoles enhanced metamorphosis and the first direct proof of a direct pituitary effect on the thyroid gland was by Smith and Smith (7) in 1922, when they demonstrated that saline extract of fresh bovine pituitary glands into hypophysectomized tadpoles reversed the thyroid atrophy and stimulated metamorphosis.

This work was confirmed by others (8) and was extended to other species. By 1932 a bioassay for thyrotropin (TSH) measuring histological changes in guinea pig thyroid glands had been developed by Junkmann and Schoeller (9).

It is now generally accepted that TSH is the major control mechanism regulating thyroid hormone secretion.

Structure

Initial attempts at isolation and purification of TSH were carried out in 1926 (10) but it was 1940 before reasonably pure (1 unit/ml) preparations were obtained (11). The next advance in purification was the use of the cation exchange resin IRC-50 chromatography in 1953 by Heideman (12). With the development of techniques in ion exchange chromatography purification to potencies greater than 20 units/mg of protein were achieved (13).

Much of the more recent work on the purification and structural analysis has come from the laboratory of Pierce using countercurrent distribution and gel filtration in the final steps of purification followed by amino-acid sequencing (14-19). With these purification techniques, human and bovine TSH preparations containing 20-40 units/mg of protein have been obtained.

TSH is a basic glycoprotein with an estimated molecular weight of 28,000. It contains 7-8% carbohydrate by weight. Like the other glycoprotein hormones it appears to be made up of an alpha and a beta chain, and in fact evidence is accumulating that the alpha chain of all the glycoprotein hormones is similar if not the same. The thyroid stimulating action of TSH appears to reside in the beta chain (20).

Regulation of TSH Secretion

The final delineation of how pituitary TSH secretion is regulated, awaited the development of a sensitive, specific and reproducible assay for TSH. The first successful attempt to assay TSH was by Junkmann and Schoeller in 1932 (9) and the J:S (Junkmann-Schoeller) unit was widely used until the USP Thyrotropin Reference Substance was established in 1951.

Before the development of a reliable radioimmunoassay for human TSH by Utiger (21) and by Odell (22) in 1965,

almost every known effect of TSH on the thyroid gland had been used as a basis for an assay of TSH. These bioassays have been well reviewed in a number of publications (23-27) and will not be further discussed here. It should be noted that it was a serendipitous observation during the development of a guinea pig bioassay for TSH which led to the discovery of the long acting thyroid stimulator (28).

Most current assays for TSH are modifications of Utiger's or Odell's radioimmunoassay (21,22) which was based on the pioneering radioimmunoassay studies of Yalow and Berson (29). The average human pituitary contains 100-600 μG of TSH and secretes approximately 110 μG per day in euthyroid adults (30). TSH secretion is controlled mainly by negative feed-back from thyroid hormones and by Thyrotropin Releasing Hormone (TRH) secreted by the hypothalamus and reaching the pituitary via the portal system of vessels.

The negative feed-back system of the thyroid-pituitary axis has been recognised since the late 1930's and was clearly delineated by Hoskins in 1949 (31). The neurohormonal hypothesis of Harris (32) and early studies of Shibusawa (33) on Thyrotropin Releasing Factor led to intensive research which culminated in the isolation, purification, structural analysis and finally synthesis of TRH by two groups simultaneously in 1970 (34,35). The intricate servo-mechanism by which the

details of thyroid hormone secretion is controlled are not yet finally worked out, but the evidence suggests that the negative feed-back of thyroxine (T_4) and triiodothyronine (T_3) (Oppenheimer and his co-workers (36) suggest that T_3 is the predominant inhibitor) is mainly at the pituitary level controlling fluctuations in the system, whereas TRH controls the set-point (37).

Numerous studies (22,23,38-40) have demonstrated that circulating levels of TSH are either low normal, low, or undetectable in hyperthyroidism due to almost any cause (exceptions being the very rare cases due to a thyrotropin producing pituitary tumour and ectopic TSH producing tumours). There is decreased or total lack of responsiveness to intravenous TRH administration in hyperthyroid patients, and Hall, et al (41) has demonstrated similar lack of response to TRH even in euthyroid Graves' disease patients. In hypothyroidism due to primary failure of the thyroid gland, TSH levels are elevated (10-1000 fold) and are further increased by exogenous TRH. Recently the syndrome of hypothyroidism due to primary hypothalamic failure (? tertiary hypothyroidism) has been described (42).

2. THE LONG ACTING THYROID STIMULATOR (LATS) AND THE PATHOGENESIS OF GRAVES' DISEASE

The disorder commonly known in the English-speaking world as Graves' disease was first described by that physician in 1835 (43). The original description has been variously ascribed to Parry (first case described 1786 but only published in 1825) (44), Flajani (1802) (45) and von Basedow (1840) (46). Werner (47), who recently reviewed the various original descriptions, suggests that a fairer eponym would be P-G-B or Parry-Graves-Basedows' disease. By 1908 the literature was already so great on this disease that Sattler devoted 152 pages of his classical 605 page monograph "Basedow's Disease" (48) to his bibliography - a total of 3,210 references.

Many theories regarding the etiology of this condition were put forward but most were displaced in the 1920's with the discovery of thyrotropin and the pituitary thyroid axis. Since thyrotropin stimulated the thyroid gland and since it was early discovered that thyrotropin also produced exophthalmos in animals (49-52), it seemed natural to assume that excess TSH was the cause of Graves' disease. By the mid 1950's as was pointed out by Means (53) the putative etiologic role of TSH in Graves' disease was becoming increasingly untenable in view of consistent inability to detect increased circulating TSH and evidence that pituitary thyrotrophs were, in fact, involuted in hyperthyroidism.

In 1956 Adams and Purves (28) published the observation that the serum of some patients with Graves' disease when injected into prepared guinea pigs, exhibited a delayed stimulatory effect on the thyroid when compared to TSH. McKenzie, in the process of developing a bioassay for TSH using the discharge of labelled thyroid hormones from mouse thyroid gland as the end point, soon confirmed this finding (54). The McKenzie mouse bioassay or some modification of it is now the world-wide standard method for detecting and measuring this substance which became known as the Long Acting Thyroid Stimulator (LATS). Since its discovery 17 years ago, considerable attention has been paid to the role of this substance in the pathogenesis of the cardinal manifestations of Graves' disease, but as yet (1973), the picture is far from clear.

LATS was soon shown to be an IgG or 7s gammaglobulin (55) unrelated to the pituitary gland (56-58), and in fact it is a product of lymphocytes (59-60). The work of Meek (61) recently partially confirmed by others (62) demonstrating that the LATS effect can be mimicked by bovine TSH-antibody complexes is difficult to reconcile with current theories of LATS production. By current methods it is not possible to obtain much purification of LATS from the mass of other IgG's

present in serum and since it has been shown by many authors to be inhibited and absorbed by various thyroid cell preparations in an antigen-antibody type of reaction, it has become accepted that LATS is an antibody to some thyroid cell component (63-65). The thyroid antigen has not been isolated but there is general agreement that it resides in soluble fractions of broken cell preparations (66-68). LATS activity can also be inhibited by antiserum to human IgG (55) by antisera to both kappa and lambda chains of human IgG (69), and antisera to Fc or Fab fragments of human IgG (70). There is no animal model for Graves' disease, despite early hopes that injection of animals with thyroid homogenates would produce LATS (71-73), this has not been substantiated by later work (74,75).

The role of LATS in the pathogenesis of the diagnostic triad (hyperthyroidism, ophthalmopathy and dermopathy) is as yet unclear and is currenlty a matter of greater controversy than ever before.

The early evidence that LATS was responsible for tne hyperthyroidism included - a) evidence that infusions of LATS-IgG stimulated the human thyroid gland (76); and b) the correlation of neonatal Graves' disease with LATS in the child's serum (77,78). Volpe (79,80) and Chopra and Solomon (75,81-83) have recently suggested that LATS has little, if any, role to play in the hyperthyroidism. This

view is based mainly on (a) the inability to detect LATS by the standard mouse bioassay in more than 45% of unconcentrated, or more than 70% of concentrated Graves' disease sera (84,85), and (b) the lack of correlation between LATS and thyroid suppressibility reported by some authors (81,82,86,87). Volpe has demonstrated a defect in cell mediated immunity in Graves' disease (80) and has reported an increased proportion of thymic derived lymphocytes in the circulation of Graves' disease patients (88) and Solomon has stated (75) that he feels the disorder is either one of defective cell-mediated immunity or an intrathyroidal defect with autoimmune epiphenomena.

McKenzie, however, is still of the view that LATS causes the hyperthyroidism of Graves' disease (89). He believes that failure to detect LATS in a higher proportion of patients is due to insensitivity of the bioassay. He admits the problem of lack of correlation of clinical features and suppressibility with LATS is more difficult to explain but suggests that a "permissive factor" is required at the cellular level in order for LATS to be active.

Early work led to the suggestion that LATS was the cause of the ophthalmopathy in Graves' disease (65,90,91). This has not been confirmed. McKenzie has reviewed the evidence for

and against a LATS ophthalmogenic effect (92). There is recent evidence from several laboratories that another immunoglobulin, distinct from LATS, is present in the blood of patients with Graves' disease, which may be responsible for exophthalmic effect (93-95). It is difficult to correlate these findings with the early findings relating TSH to experimental exophthalmos and the more recent work of Kohn and Winand demonstrating that proteolytic digestion of TSH separates the thyroid stimulating and exophthalmogenic activities of that substance (96,97). They have demonstrated that the exophthalmogenic activity resides mainly in a component consisting of the beta chain plus the amino-terminal part of the alpha chain. Similarly, the finding of Mahieu (98) that a defect of cellular immunity to retro-orbital tissue is present in Graves' disease remains unelucidated.

The earlier findings that LATS presence and levels correlate best with the presence of pretibial myxoedema (reviewed in Ref. 65) have also been questioned by later reports (99,100).

Thus, although the very early observation that Graves' disease involved the thymic-lymphoid-immune system (53) have been corroborated, the roles of the humoral and cellular systems in the pathogenesis of this disorder have yet to be defined.

3. ACTIONS OF THYROTROPIN AND THE LONG-ACTING THYROID STIMULATOR

TSH and LATS have been shown to have many actions in common on the thyroid gland. The more important of these are summarised (with appropriate references) in Table I. The major differences in effects between the two thyroid stimulators include minor quantitative differences in their effects on thyroidal lipid synthesis (113) and the inability of LATS to stimulate the chick thyroid gland (120). Also D'Angelo was unable to detect any effect of Graves' disease serum in the stasis tadpole assay (121).

A number of extrathyroidal effects of thyrotropin have been reported. The earliest reported and best known of these effects is the exophthalmos producing effect (49,50). Attempts to separate the thyroid stimulating and exophthalmos producing effects of the earlier crude TSH preparation, led to conflicting results (122-124). The highly purified mouse tumour TSH prepared by Bates, et al. (125) had no exophthalmogenic effect, yet this data conflicts with the more recent findings of Kohn and Winand already quoted (96,97).

TSH effects on lipid metabolism and adipose tissue will be discussed in the following section.

There have been two reports of an effect of TSH on thyroxine binding proteins independent of thyroid function.

TABLE I

TSH AND LATS ACTIONS ON THYROID GLAND

ACTION	TSH REFERENCE	LATS REFERENCE
Glucose Metabolism		
↑ Glucose Uptake	101	113
↑ Hexose Monophosphate Shunt	102	113
↑ Glycolytic Path	102	113
↑ Oxygen Uptake	101	113
↑ Lactate Production		113
Pyridine Nucleotides		
↑ NADP	103	103
↓ NAD	103	103
Phospho Lipids		
↑ Phospholipid Synthesis	104	113
↑ Phosphatidic Acid	105	
↑ Phosphatidyl Inositol	105	
↑ Lecithin		113
Lipids		
↓ Triglyceride/Diglyceride Ratio	106	
↓ T/D Ratio		113
↑ Lipid Synthesis		113

ACTION	TSH REFERENCE	LATS REFERENCE
Iodoprotein Synthesis		
↑ Iodine Transport	107	114
↑ Protein Incorporation	108	115
↑ T_3/T_4 Ratio	109	116
↑ Iodine Organification	110	116
Thyroid Hormone Secretion		
↑ Colloid Droplets	111	117
↑ PBI^{131}	90	90
↑ Thyroxine Release	112	116
Cyclic AMP		
↑ Cyclic AMP Production	118	119

Sisson (126) reported a decrease in thyroidectomized guinea pig thyroxine binding proteins, but could not demonstrate a similar effect in one athyreotic man. Banos and Tako (127) report some inconclusive evidence that TBG is increased by TSH.

There had been no reports of extrathyroidal effects of LATS prior to the work prescribed in this thesis. The data presented in this thesis and in particular that published in the first paper (128) was the first such report.

4. HORMONAL EFFECTS ON ADIPOSE TISSUE

Adipose tissue, like bone, was, until the early 1950's, considered to be a relatively inert tissue. Over the past 20 years it has become evident that it is, in fact, a very active tissue metabolically and produces the body's second most important energy source in the form of free fatty acids (FFA).

The major role of adipose tissue is the storage of energy in the form of triglycerides and the release of this potential energy source as FFA (lipolysis). The precursors of the stored triglyceride are glucose and FFA. Although the brown fat possessed by some animals contains large amounts of glycogen as a glucose source, in white adipose tissue it appears that most, if not all, the glucose for lipogenesis comes from the

circulation (129). Adipose tissue is known to contain at least 3 isoenzymes of hexokinase (130,131) responsible for the phosphorylation of glucose. The steps involved in the conversion of glucose to lipid in adipose tissue are the same as the first part of glycolysis, down to triose phosphate (132). Dihydroxyacetone phosphate obtained by the aldolase step in glycolysis is converted to glycerol phosphate which is the major acceptor for long chain fatty acids in the synthesis of neutral lipids.

The origin of the fatty acids stored in human adipose tissue is still in dispute (133). The three sources are a) fatty acid synthesis from acetyl-CoA, b) fatty acids obtained from the circulation by the action of lipoprotein lipase and c) fatty acids released by intracellular lipolysis. The contributions made by each source vary with metabolic status of the animal at any given time.

Lipolysis is achieved in the adipocyte by a group of intracellular enzymes called lipases. As many as 17 esterases have been identified in human adipose tissue by starch gel electrophoresis (134) but probably there are only two groups of intracellular lipases (135). These are a triglyceride lipase and a monoglyceride lipase. In the rat there may also be a diglyceride lipase (136). The triglyceride lipase appears to be the rate limiting enzyme in lipolysis and is sensitive to

the action of many hormones, hence the term often applied to it - hormone sensitive lipase. The fatty acids released are either re-esterified with glycerol phosphate obtained from glycolysis or escape from the cell and are transported to the liver bound to albumin. Most of the glycerol released is returned to the liver via the circulation, but contrary to previous reports (137) a small amount is re-esterified via glycerokinase action (138,139).

The first demonstration of an effect of hormones on adipose tissue was by Best and Campbell in 1936 (140). Lipid mobilisation to skeletal muscle from liver and adipose tissue by crude TSH was first demonstrated by Dobyns in 1946. The great advances in adipose tissue metabolism over the past 20 years has depended on the development of improved methods of lipid analysis, metabolic techniques (especially the use of radioactive isotope tracers) and in vitro techniques for handling adipose tissue (141).

Since the early in vitro demonstrations of hormonal effects on adipose tissue (142,143,144) many hormones have been shown to effect adipose tissue metabolism. Hormones which effect adipose tissue metabolism with some interspecies variation include insulin, glucagon, corticosteroids, catecholamines, growth hormone, ACTH, TSH, prostaglandins, thyroid hormones and secretin. These have been well reviewed (133,141).

The first reports of an in vitro effect of TSH on adipose tissue were by White and Engel (144) and Freinkel (145). They demonstrated that TSH stimulated oxygen uptake and glucose assimilation and enhanced lipolysis in rat epididymal adipose tissue. The lipolytic effect of TSH on guinea pig adipose tissue was demonstrated by Rudman (146) who found this tissue relatively less sensitive than that of the rat. In vitro lipolytic effects of TSH have also been demonstrated on adipose tissue from dogs and mice (147) and more recently from man (148). An earlier study (149) had failed to demonstrate such an effect on human adipose tissue in vitro and one study (150) failed to demonstrate a lipolytic action in man in vivo. Although most of the experiments used bovine TSH, Fernie and co-workers (151) demonstrated a similar effect using highly purified human TSH on rat adipose tissue.

A number of studies have looked at the effects of thyroxine and triiodothyronine on adipose tissue in vitro and on the effects of experimental hyperthyroidism and hypothyroidism on adipose tissue metabolism. Hagen (152) demonstrated on increased uptake of oxygen and enhanced sensitivity to insulin in adipose tissue from hyperthyroid rats. In induced hypothyroidism in animals there is decreased oxygen utilisation and decreased sensitivity of that parameter to epinephrine (153). Lipolysis and its stimulation by epinephrine is enhanced in tissue taken from

hyperthyroid animals and decreased or absent in hypothyroid tissue. Brodie (154) has presented evidence to suggest that this effect is due to changes in the amount of the adenyl cyclase system available to the lipolytic hormone. No direct effects of T_4 or T_3 have been demonstrated on adipose tissue in vitro.

COMPARISON OF THE EFFECTS OF THYROTROPIN AND THE LONG-ACTING THYROID STIMULATOR ON GUINEA PIG ADIPOSE TISSUE

BY

I. R. HART, and J. M. McKENZIE

Since its discovery in 1956 (1), the long-acting thyroid stimulator (LATS) has been the subject of investigation particularly regarding its role in the pathogenesis of Graves' disease and its relationship to thyrotropin (TSH). Despite well-established chemical differences both substances have many qualitatively similar, if quantitatively and temporally different, actions on the thyroid gland. These have been reviewed in several recent publications (2-4).

White and Engel (5) were the first to show an in vitro lipolytic effect of TSH, and Freinkel (6) found that TSH enhanced oxygen consumption and glucose utilization, and stimulated the release of free fatty acids (FFA), by rat adipose tissue. These observations have been confirmed many times and a lipolytic effect of TSH was shown in fat slices and, in some cases, with isolated fat cells, from adipose tissue of guinea pigs, dogs and mice, as well as rats (7, 8).

In view of the similarity in thyroid actions of TSH and LATS, we compared their effects on glucose metabolism and FFA release in guinea pig adipose tissue by measuring in vitro lipolysis and the conversion of U-^{14}C-glucose to $^{14}CO_2$ and its incorporation into both the total and the fatty acid moiety of neutral lipids.

MATERIALS and METHODS

Male guinea pigs (600-900 g) obtained from Quebec Breeding Farm, St. Eustache, Quebec, were maintained at a constant ambient temperature and fed Purina Chow and water ad lib. for at least 10 days before use. The animals were killed by decapitation under light ether anesthesia and the distal parts of the epididymal fat pads were excised and randomly pooled in 3 Petri dishes containing 0.9% NaCl solution. The tissue from 3-6 animals was used in any one experiment. The fat pads were cut into pieces of 50-150 mg, and 3 or 4 pieces totaling 200-400 mg were added to each incubation flask, a 25 ml Erlenmeyer flask with center well.

Incubations were carried out for 2 hr at 37C in Krebs-Ringer bicarbonate (KRB), pH 7.4, containing glucose 100 mg/100 ml, 4% bovine serum albumin (BSA) and, in some experiments, 0.5 or 1.0 μCi U-^{14}C-glucose. In experiments where $^{14}CO_2$ was measured, an 8 x 2 cm strip of Whatman No. 1 filter paper was placed in the center well and 0.2 ml of 10X Hyamine was added to it 10 min before the end of incubation. For incubations the flasks were stoppered and gassed for 5 min with a 95% O_2-5% CO_2 mixture and then placed in a Dubnoff metabolic incubator shaking at 80 strokes/min. Reactions were terminated by the addition of 0.2 ml 2N HCl or, in experiments where lipolysis alone was measured, by the removal of the tissue from the medium. In appropriate experiments, the Hyamine-soaked filter

paper was removed 15 min after the end of incubation and the radioactivity was counted in 15 ml of scintillation fluid with 3 drops of ethanol added. The tissue was removed and, after careful rinsing, total glycerides were extracted (9) and an aliquot was dried, weighed, dissolved in scintillation fluid and the radioactivity was counted. Another aliquot was saponified in 10% KOH in ethanol at 90 C for 90 min. The fatty acids released were extracted in heptane and an aliquot of this was evaporated, weighed and the content of ^{14}C was measured. Free fatty acid release was estimated by the Dole method (10) using 2 ml of the incubation medium.

U-^{14}C-glucose of specific activity 1.6-1.7 mCi/mg was obtained from the Radiochemical Centre, Amersham, Bucks., England.

Thyrotropin was either NIH-TSH-B4 or Thytropar (Armour Pharmaceutical Co., Chicago, Ill.), lot D9812. Rabbit antiserum to TSH was prepared by the intramuscular injection of 1 U Thytropar, mixed with Freund's complete adjuvant, at intervals of 5 days for 5 doses. The antiserum was positive at a dilution of 1 in 50,800 when measured in a hemagglutinating system.

LATS-IgG was obtained from appropriate human serum by precipitation with ammonium sulfate followed by DEAE-Sephadex chromatography. Where the response to LATS was to be measured, amounts of the lyophilized IgG giving up to 1000% response in the mouse bioassay (11) were added to each flask. The same

amounts of human IgG (Immunology Inc., Lombard, Ill.) were added to control flasks. Horse antiserum to human IgG (Hyland, lot 8401D003Ai), 0.2 ml in each flask, was used in the LATS neutralization test.

Microsomes were obtained from pooled normal thyroid glands and portions of liver obtained at autopsy. The tissues were homogenized in 4 vol of 0.25M sucrose using a Virtis 23 blender. Following sieving through coarse gauze, further homogenization was performed in a teflon Potter-Elvehjem homogenizer. Discarding the cellular debris, mitochondria and nuclei sedimenting at 8000 g, the microsome pellets were obtained by centrifugation at 105,000 g for 2 hr. The thyroid and liver microsome pellets were homogenized by hand in 0.9% NaCl containing appropriate amounts of LATS-IgG and incubated overnight at 5 C. The microsomes were then re-sedimented at 105,000 g for 2 hr and discarded, and the supernatant solution was lyophilized after 8 hr dialysis against distilled water. A gram-equivalent (gEq) of microsomes was that obtained from 1 g of starting material.

All scintillation counting was performed in a Packard Tri-Carb liquid scintillation counter, model 4312, using 15 ml of scintillation fluid consisting of 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis- 2-(5-phenyloxazolyl) -benzene dissolved in toluene. The statistical significance of differences of means was established by use of Student's t test.

RESULTS

Lipolysis. Lipolysis was consistently enhanced by concentrations of TSH of 0.01 mU/ml or greater, the peak effect being seen with 10 mU/ml (Fig. 1). In one experiment a lipolytic effect was seen with 0.001 mU/ml. LATS-IgG enhanced lipolysis in concentrations of 0.166 mg/ml (0.5 mg/flask) or more, the greatest effect being seen at a concentration of 0.666 mg/ml (2 mg/flask) (Fig. 2). These flask amounts, when injected per mouse in the LATS bioassay, produced responses of 158 and 396%, respectively. In other experiments (not shown) normal human IgG, <2mg/ml, had no influence on lipolysis.

The addition of 0.1 ml of rabbit antiserum to TSH did not affect basal lipolysis or LATS-induced lipolysis but completely inhibited TSH-induced lipolysis. Similarly, adding 0.2 ml of horse antiserum to human IgG had no effect on basal lipolysis or on TSH-induced lipolysis, but significantly reduced the lipolytic effect of LATS (Fig. 3); this was a finding confirmed in two other experiments.

As shown in Fig. 4, preincubation of the LATS-IgG with thyroid microsomes, 10 gEq/flask, completely inhibited the lipolytic effect, whereas preincubation with a similar amount of liver microsomes had no significant effect. On conventional bioassay for LATS (11) there was, similarly, inhibition of LATS by thyroid microsomes but not by liver microsomes.

Glucose metabolism (Table 1). Neither TSH nor LATS significantly enhanced conversion of ^{14}C-glucose to $^{14}CO_2$ nor its incorporation into total glyceride. Both substances, however, caused decreased incorporation of ^{14}C from glucose into glyceride fatty acids.

DISCUSSION

The results reported here indicate that LATS, like TSH, has extrathyroid effects in that it increases lipolysis and decreases glucose conversion to fatty acids in guinea pig adipose tissue.

There are several studies comparing the effects of TSH and LATS on thyroid metabolism in vitro. Scott and co-workers (12) studied the effects of the two stimulators on sheep thyroid slices and showed that both stimulated glucose uptake and oxidation, and lactic acid production, to a similar degree. Both substances enhanced ^{32} P-orthophosphate incorporation into total phospholipids but differences were noted in incorporation into individual phosphatides. Further, unlike TSH, LATS consistently stimulated U-^{14}C-glucose incorporation into total neutral lipids though both had a similar effect in decreasing the triglyceride/diglyceride labelling ratio. It was suggested that these differences in the actions of TSH and LATS indicated they might share some common sites of action, yet differ in others. Field and his colleagues (13) obtained, in part,

similar results with canine thyroid slices and further showed that the peak response to LATS was several hours later than that to TSH. They noted an inconstant stimulatory effect of LATS on glucose oxidation by spleen and liver slices. More recently, Burke (14) confirmed the stimulatory effects of TSH and LATS on sheep thyroid slice glucose oxidation and phospholipogenesis, but found no latency of LATS effects when they were compared to effects of TSH.

In the present studies TSH and LATS had similar actions on guinea pig adipose tissue. Both thyroid stimulators were lipolytic and both decreased the proportion of glucose carbons being incorporated into glyceride fatty acids. The small amounts of protein (0.166 mg/ml) as LATS-IgG necessary to enhance lipolysis in these studies compare well with the amounts used for in vitro thyroid studies with this substance and the specificity of the action has been demonstrated by its inhibition by those manipulations, namely, with anti-IgG serum and thyroid microsomes, that specifically inhibit LATS in bioassay. (2-4).

TSH is said not to be lipolytic on human adipose tissue in vitro (15), nor does it appear to be so in vivo in man (16). We have no data relevant to a lipolytic effect of LATS on human tissue and therefore may draw no conclusions regarding the clinical consequence of lipolysis by LATS.

LATS not only is an IgG but has tentatively been identified as an antibody to a thyroid cell component (2-4). This concept, that an antibody can have stimulatory effects on cell metabolism, is further strengthened by the present studies. There is speculation that LATS may act by inactivating an inhibitor of thyroid function (2) and it may be that a similar inhibitor exists in guinea pig adipose tissue.

While the identity of the putative antigen homologous to LATS is uncertain, there are indications that adenyl cyclase or its product, cyclic adenosine 3',5'-monophosphate, is the mediator of both TSH (17, 18) and LATS (19, 20) actions on the thyroid. The same enzyme system has been implicated as mediating the activation by TSH and other hormones of the lipase in adipose tissue (21). It may be, therefore, that the adenyl cyclase-cyclic adenosine 3',5'-monophosphate system is involved also in mediating the effects of LATS we described here. This is at present under investigation.

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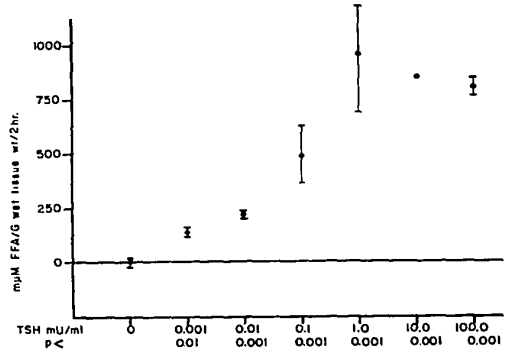

FIG. 1. Effect of TSH on lipolysis in guinea pig adipose tissue; dose-response relationship. Data are shown as the mean ±SD of triplicate observations.

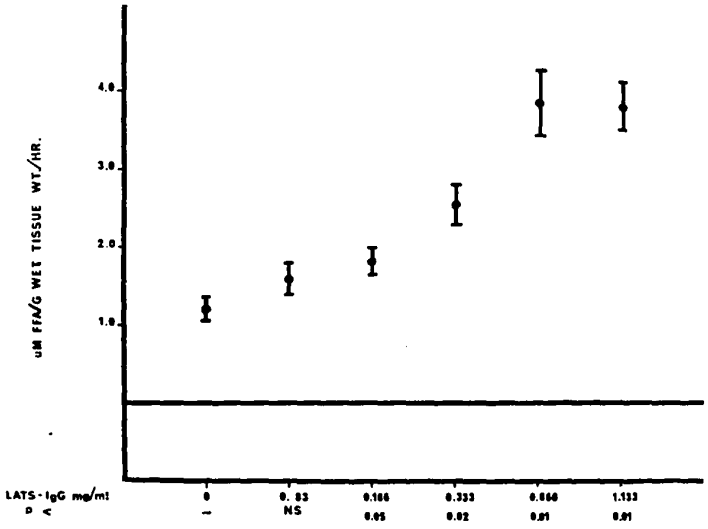

FIG. 2. Effect of LATS-IgG on lipolysis in guinea pig adipose tissue; dose-response relationship. Data are shown as the mean±SD of triplicate observations.

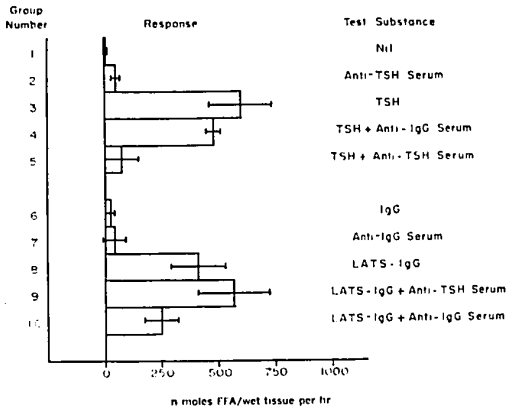

FIG. 3. Inhibition of TSH- and LATS-induced lipolysis in guinea pig adipose tissue by specific antisera. TSH was tested at 100 mU/ml, normal and LATS-IgG at 3.5 mg/ml, anti-TSH serum at 0.1 ml/flask and anti-IgG serum at 0.2 ml/flask. p values for significance of differences of means are as follows:

Groups compared	p	Groups compared	p
1 *vs.* 2	NS	6 *vs.* 7	NS
1 *vs.* 3	<0.001	6 *vs.* 8	<0.001
3 *vs.* 4	NS	8 *vs.* 9	NS
3 *vs.* 5	<0.001	8 *vs.* 10	<0.05
4 *vs.* 5	<0.001	9 *vs.* 10	<0.01

TABLE 1. Effects of TSH and LATS on the metabolism of U-^{14}C glucose in guinea pig epididymal adipose tissue

	Control	TSH 100 mU ml	p	Control IgG 2.5 mg/ml	LATS IgG 2.5 mg/ml	p
Converted[1] to CO_2	330 ± 93	427 ± 130	NS	337 ± 98	431 ± 122	NS
Incorporated[1] into total lipid	524 ± 142	767 ± 226	NS	563 ± 156	727 ± 217	NS
Incorporated into glyceride FA	357 ± 35[2]	148 ± 16	<0.001	213 ± 17[3]	87 ± 14	<0.001

Results expressed as nmoles of glucose per g of wet tissue weight per hr of incubation. Values shown are means of pooled data ± SE.

[1] Pooled data from 2 experiments (6 observations in each).

[2] Pooled data from 4 experiments (triplicate observations).

[3] Pooled data from 2 experiments (5 and 4 observations).

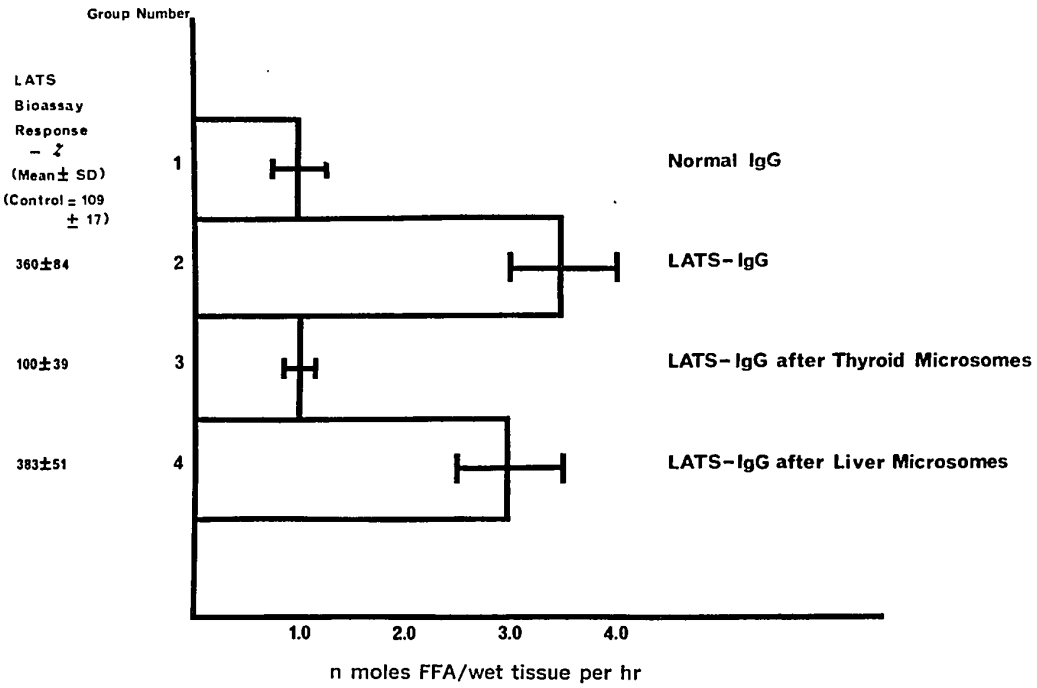

FIG. 4. Effect of preincubation with thyroid and liver microsomes on the lipolytic activity of LATS-IgG. The data are shown as the mean ± SD of triplicate observations. p values for significance of differences of means are as follows:

Groups compared	p
1 *vs.* 2	<0.001
2 *vs.* 3	<0.001
2 *vs.* 4	NS

LATS activity in preparations 2, 3, and 4 was assayed in mice (11) and the responses are listed as % increases in blood radioactivity (mean ± SD); control solution was 1% human serum albumin in 0.9% NaCl.

The most striking and most reproduceable effect that the two stimulators had in common was enhancement of lipolysis. The preceding paper discussed this only in relation to guinea pig adipose tissue. That which follows extends these observations to other species, in particular demonstrating a similar effect on human adipose tissue.

THE LIPOLYTIC RESPONSIVENESS OF ADIPOSE TISSUE OF VARIOUS SPECIES TO THYROTROPIN AND THE LONG-ACTING THYROID STIMULATOR

BY

IAN R. HART

M.B., Ch.B., F.R.C.P.(C)

Previous studies from this laboratory confirmed the work of others that thyrotropin (TSH) was lipolytic when added to guinea pig adipose tissue in vitro and demonstrated for the first time a similar effect of the long-acting thyroid stimulator (LATS) (1). Our work with other species tends to be at variance with that of Rudman who demonstrated relative insensitivity of guinea pig adipose tissue to TSH when compared to that of the rat (2). There are also conflicting results regarding the responsiveness of human adipose tissue to bovine TSH in that Raben (3), and Mosinger (4) could demonstrate no TSH lipolytic effect on human adipose tissue either in vitro or in vivo, whereas, Bray and Trygstad (5) demonstrated an in vitro effect of bovine TSH on human adipose tissue.

This paper presents dose response curves for both TSH and LATS-IgG on adipose tissue from guinea pig, rat, mouse and man. It confirms that bovine TSH has a lipolytic effect on human adipose tissue and demonstrates for the first time that purified LATS-IgG preparations are also lipolytic when added to human adipose tissue in vitro.

MATERIALS AND METHODS

TISSUES

Albino guinea pigs (600-900 g), hooded rats (300-400 g) and albino mice (25-30 g) were maintained under stable conditions for 10 days prior to sacrifice. They were killed

by a blow to the head under light ether anesthesia. Tissues were removed immediately after death, sliced and pooled in cold 0.9% NaCl solution. In most experiments epididymal adipose tissue was used. In some experiments guinea pig subcutaneous and perinephric adipose tissue and liver was used.

Human omental adipose tissue was obtained from non-obese non-diabetic patients at laparotomy. It was immediately sliced and place in cold 0.9% NaCl solution.

In some experiments 2-4 slices totaling 150-300 mg were added to the incubation medium. In other experiments free fat cells were prepared by the method of Rodbell (6) and 0.2 ml of the packed free fat cells was added to each flask.

MATERIAL

TSH was NIH-TSH-B4. Normal pooled human IgG and LATS-IgG were prepared from the appropriate sera by precipitation with 40% ammonium sulfate followed by DEAE-Sephadex chromatography according to the method of Kriss et al. (7). Human TSH standard H-TSH68/38 was supplied by the National Institute for Medical Research, London. The Standard LATS preparation was long-acting thyroid stimulator (65/122) supplied by the National Institute for Medical Research, London.

METHODS

Incubations were carried out in 3 ml of Krebs-Ringer bicarbonate (KRB) at pH 7.4 containing 4% bovine serum albumin (BSA) and glucose 100 mg/100 ml. The incubation period was 3 hr. Incubations were carried out in a Dubnoff metabolic incubator at 37°C, the gas phase was air. At the end of incubation free fatty acids (FFA) by the method of Dole (8) or glyceride-glycerol by the method of Wieland (9) were estimated in a 2 ml aliquot of the medium ± free fat cells. All groups were in triplicate. Results are expressed as µEq/G Tissue/Hour in the case of tissue slices or as µEq/flask/hour where free fat cells were used.

RESULTS

The results of the addition of bovine TSH in amounts varying from 1 µU to 100 mU/ml of incubation medium on adipose tissue from guinea pig, rat, mouse and man are shown in Figs. 1-4 respectively. Guinea pig adipose tissue was the most sensitive, significant effect being achieved with as little as 1 µU/ml and a peak effect with 1 mU/ml. Mouse adipose tissue was more sensitive to TSH than that of the rat and least sensitive of all was human adipose tissue. Human adipose tissue was somewhat more sensitive to the lipolytic effect of human TSH by the order of at least one magnitude (Fig. 5).

Only one experiment was carried out with human TSH but omental adipose tissue from several people were tested with bovine TSH with marked variability in the response. Bovine TSH had no lipolytic effect on guinea pig liver (Fig. 6).

Due to limited supplies different potent LATS-IgG preparations were used for the dose response curves on the various species. Several potent LATS-IgG preparations were tried on slices and free fat cell preparations from the mouse and none had any effect. Guinea pig adipose tissue slices responded to 0.166 mgm/ml of one LATS-IgG preparation the effect plateauing at 0.666 mgm/ml (Fig. 7). Adipose tissue from three sites in the guinea pig - epididymal, subcutaneous and perinephric - all responded significantly to both TSH and a relatively weak LATS preparation (British MRC Standard B) but the most sensitive to both stimulations was the epididymal fat (Fig. 8).

In early experiments utilising rat epididymal fat slices we had been unable to detect any lipolytic effect (10). Using a rat epididymal free fat cell preparation, however, we were able to demonstrate that rat adipose tissue was very sensitive to the lipolytic action of LATS-IgG (Fig. 9). Similarly with human omental slices no effect was detectable, whereas, human free fat cell preparation was sensitive to 0.25 mgm/ml of LATS-IgG (Fig. 10).

DISCUSSION

The first demonstration of an extrathyroidal effect of TSH in causing an enhancement of lipolysis in rat adipose tissue was by White and Engel in 1956 (11). This has since been confirmed by many workers. A similar effect on guinea pig adipose tissue was reported by Rudman et al. (12). These workers, however, found that guinea pig adipose tissue was less sensitive to TSH than that of the rat (12, 2). These results are at variance with our experience in which guinea pig adipose tissue is one thousandfold more sensitive to this thyroid stimulator than rat adipose tissue.

The data presented here confirm the results of Bray and Trygstad (5) that bovine TSH has a lipolytic effect on human adipose tissue in vitro but are at variance with those of Mosinger et al. (4). We have not looked at in vivo effects of bovine TSH in man but Raben (3) was unable to detect such an effect. Fernie et al. (13) demonstrated a lipolytic effect of purified human TSH and several fractions of this substance on rat adipose tissue, but as far as we are aware this paper is the first report of a lipolytic effect of human TSH on human adipose tissue. Fernie et al. were apparently able to separate to some degree the thyroid stimulating and lipolytic effects of human TSH, but this work has not been confirmed.

Our original results showing a lipolytic effect of purified LATS-IgG on guinea pig adipose tissue (1) has been confirmed in the rat by Kendall-Taylor and Munro using a free fat cell preparation (14). Our results using a similar preparation of rat adipose tissue confirm their observations. It is difficult to explain why rat free fat cell preparations are sensitive to LATS-IgG whereas previous work from this laboratory failed to demonstrate a similar effect on rat adipose tissue slices (10). A similar situation arises with human adipose tissue in that only free fat cells preparations are stimulated by LATS.

Although we have demonstrated that both stimulators act on adipose tissue from various sites in the guinea pig, it appears that TSH at least has no lipolytic activity on liver tissue.

The clinical usefulness of the LATS lipolytic effect on adipose tissue is as yet unclear, we have presented data elsewhere suggesting that this is a sensitive assay for the detection of LATS in unconcentrated and IgG concentrates of human sera (15). We have also, in another publication, demonstrated for the first time the presence of LATS in pleural fluid by this technique and confirmed it by the standard mouse bioassay (16).

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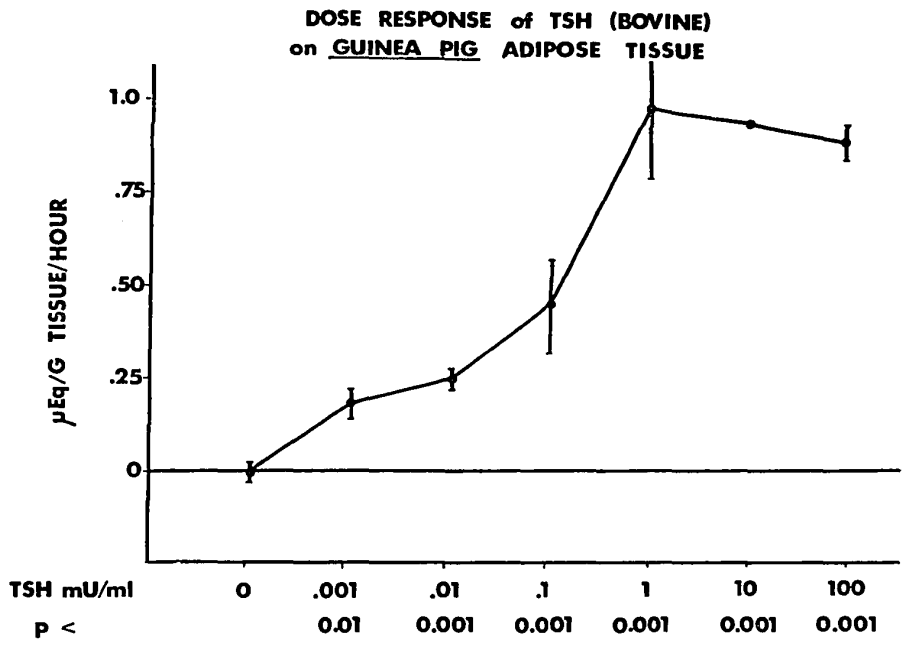
DOSE RESPONSE of TSH (BOVINE)
on GUINEA PIG ADIPOSE TISSUE
μEq/G TISSUE/HOUR
1.0
.75
.50
.25
0
TSH mU/ml
0
.001
.01
.1
1
10
100
P <
0.01
0.001
0.001
0.001
0.001
0.001

Fig.1

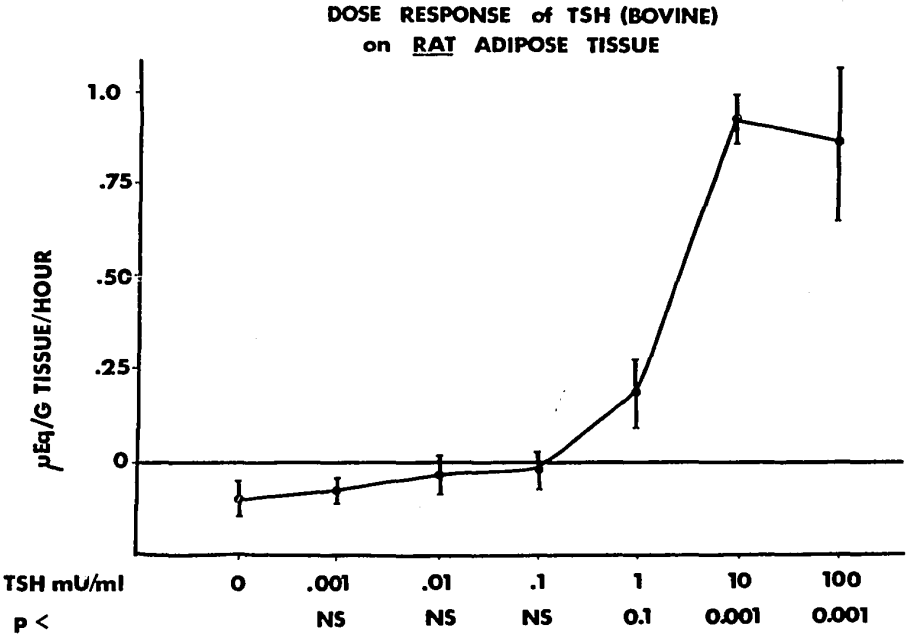
DOSE RESPONSE of TSH (BOVINE)
on RAT ADIPOSE TISSUE
μEq/G TISSUE/HOUR
1.0
.75
.50
.25
0
TSH mU/ml
0
.001
.01
.1
1
10
100
P <
NS
NS
NS
0.1
0.001
0.001

Fig.2

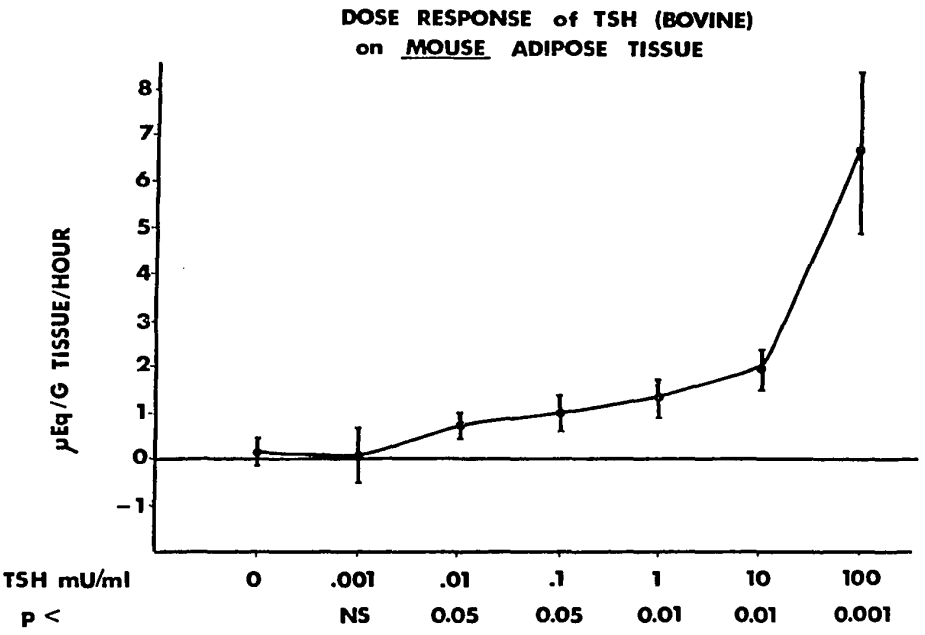
DOSE RESPONSE of TSH (BOVINE)
on MOUSE ADIPOSE TISSUE
µEq/G TISSUE/HOUR
8
7
6
5
4
3
2
1
0
-1
TSH mU/ml
0
.001
.01
.1
1
10
100
p <
NS
0.05
0.05
0.01
0.01
0.001

Fig.3

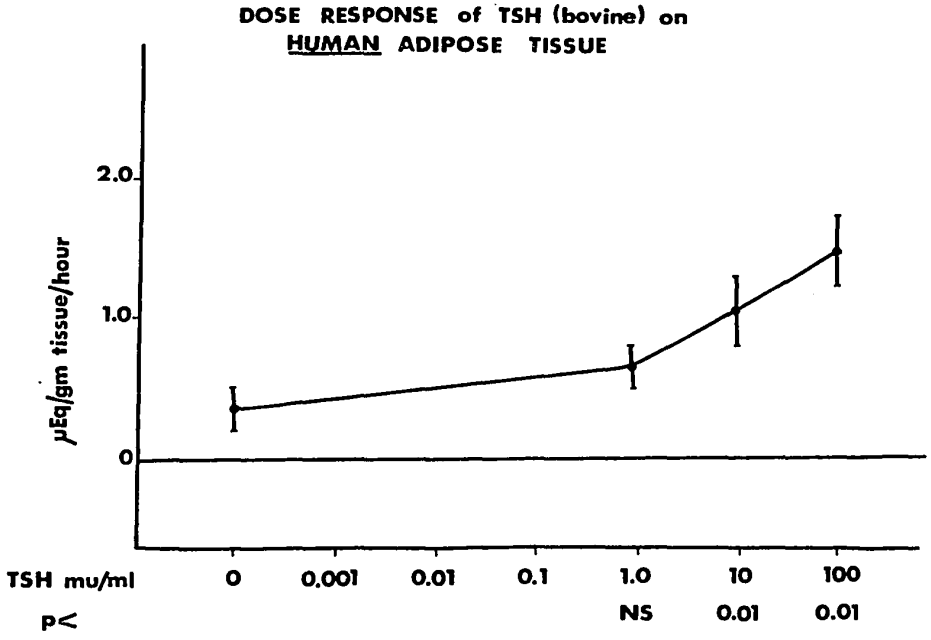
DOSE RESPONSE of TSH (bovine) on
HUMAN ADIPOSE TISSUE
µEq/gm tissue/hour
2.0
1.0
0
TSH mu/ml
0
0.001
0.01
0.1
1.0
10
100
p<
NS
0.01
0.01

Fig.4

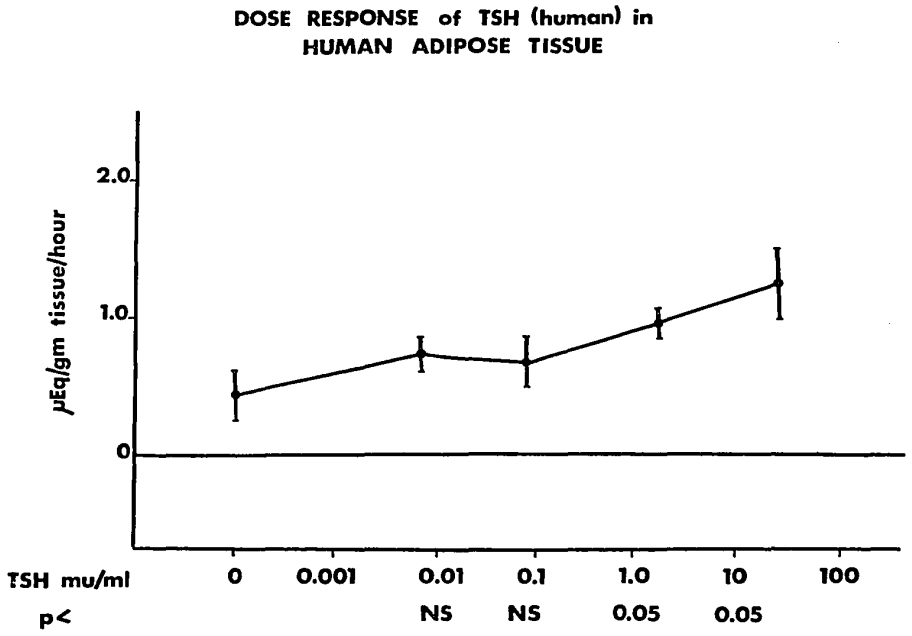
DOSE RESPONSE of TSH (human) in
HUMAN ADIPOSE TISSUE
µEq/gm tissue/hour
2.0
1.0
0
TSH mu/ml
0
0.001
0.01
0.1
1.0
10
100
p<
NS
NS
0.05
0.05

Fig.5

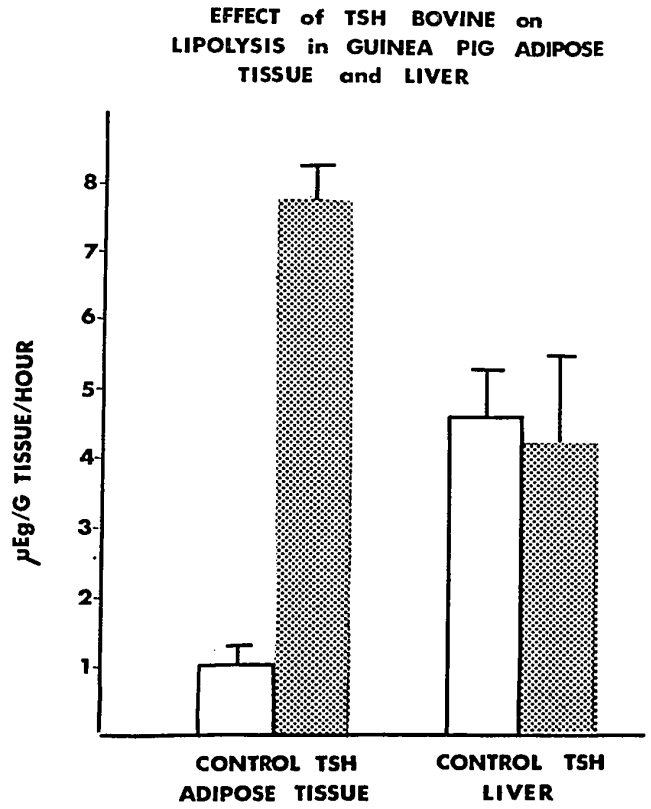
EFFECT of TSH BOVINE on
LIPOLYSIS in GUINEA PIG ADIPOSE
TISSUE and LIVER
µEg/G TISSUE/HOUR
8
7
6
5
4
3
2
1
CONTROL TSH
ADIPOSE TISSUE
CONTROL TSH
LIVER

Fig.6

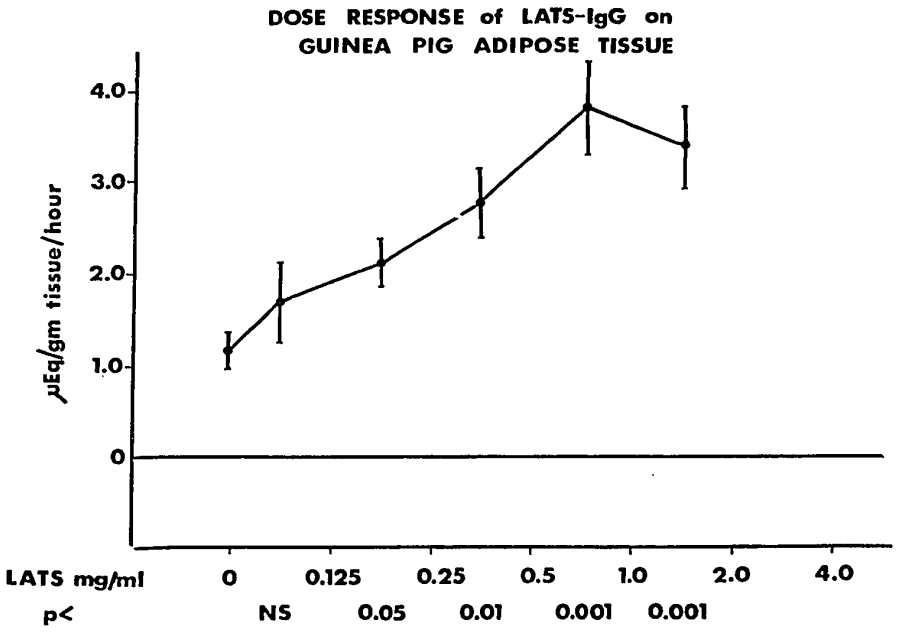
DOSE RESPONSE of LATS-IgG on
GUINEA PIG ADIPOSE TISSUE
µEq/gm tissue/hour
4.0
3.0
2.0
1.0
0
LATS mg/ml
0
0.125
0.25
0.5
1.0
2.0
4.0
p<
NS
0.05
0.01
0.001
0.001

Fig.7

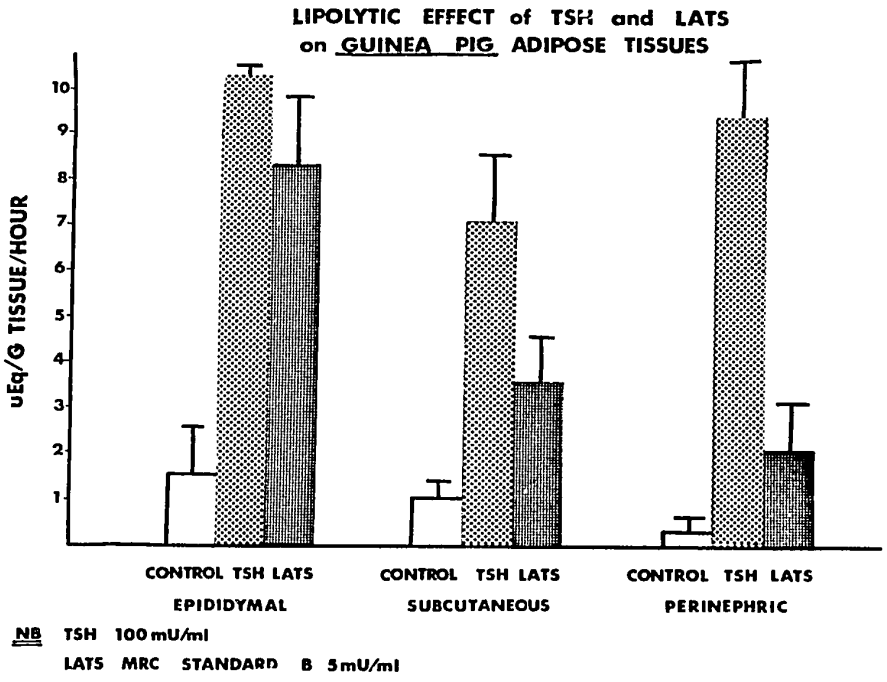
LIPOLYTIC EFFECT of TSH and LATS
on GUINEA PIG ADIPOSE TISSUES
uEq/G TISSUE/HOUR
10
9
8
7
6
5
4
3
2
1
CONTROL TSH LATS
EPIDIDYMAL
CONTROL TSH LATS
SUBCUTANEOUS
CONTROL TSH LATS
PERINEPHRIC
NB TSH 100 mU/ml
LATS MRC STANDARD B 5mU/ml

Fig.8

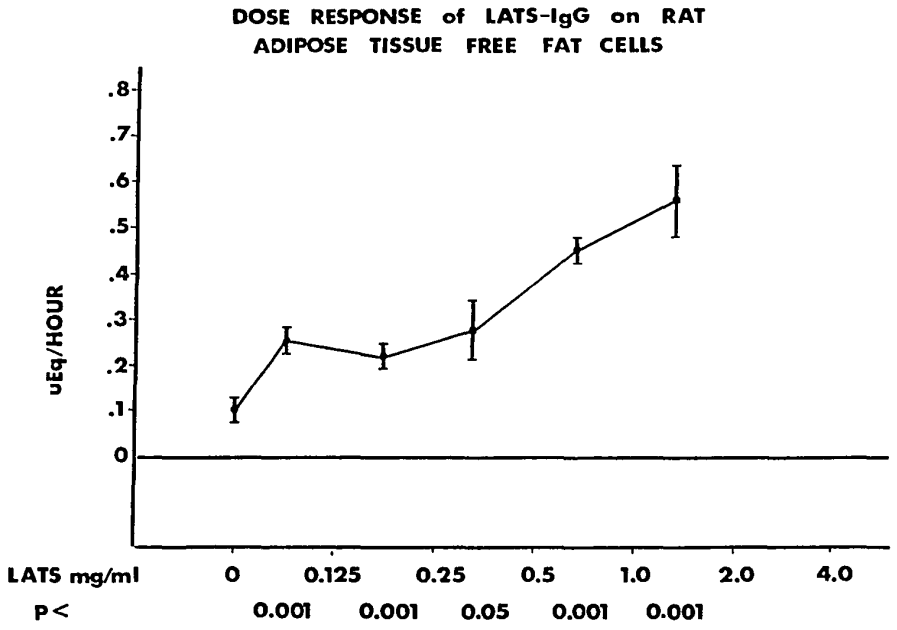
DOSE RESPONSE of LATS-IgG on RAT
ADIPOSE TISSUE FREE FAT CELLS
uEq/HOUR
.8
.7
.6
.5
.4
.3
.2
.1
0
LATS mg/ml
0
0.125
0.25
0.5
1.0
2.0
4.0
P<
0.001
0.001
0.05
0.001
0.001

Fig.9

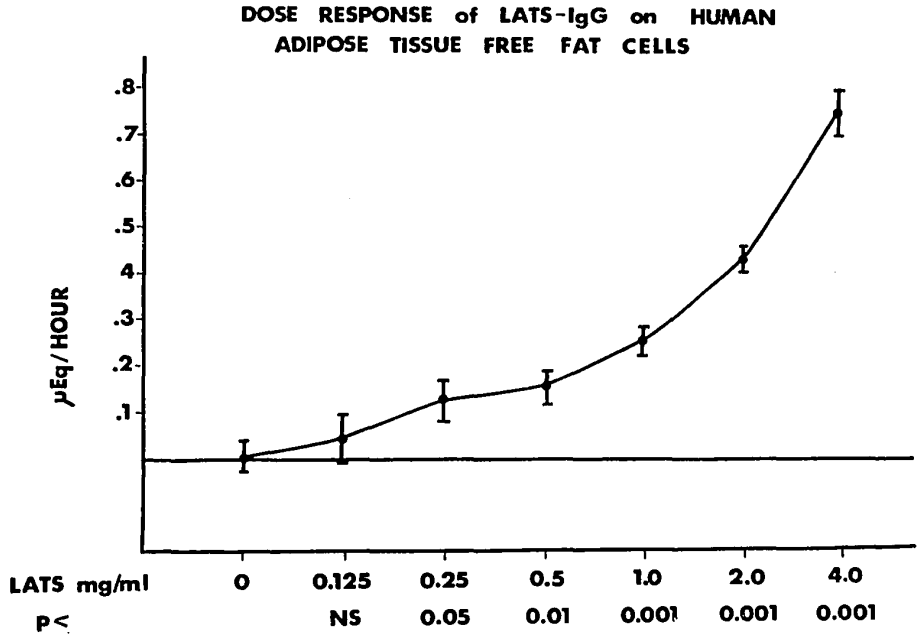
DOSE RESPONSE of LATS-IgG on HUMAN
ADIPOSE TISSUE FREE FAT CELLS
µEq/HOUR
.8
.7
.6
.5
4
.3
.2
.1
LATS mg/ml
0
0.125
0.25
0.5
1.0
2.0
4.0
P<
NS
0.05
0.01
0.001
0.001
0.001

Fig.10

Having established that LATS like TSH had a lipolytic effect on adipose tissue of several species an attempt was made to compare the time sequence of its action with that of TSH and to see if its mode of action was similar to that established for TSH.

CHARACTERISATION OF THE LIPOLYTIC EFFECTS OF THYROTROPIN AND THE LONG-ACTING THYROID STIMULATOR

BY

IAN R. HART

M.B., Ch.B., F.R.C.P.(C)

Although quite different both chemically and immunologically the two thyroid stimulators - thyrotropin (TSH) and the long-acting thyroid stimulator (LATS) - have been shown to have many actions on the thyroid gland in common (1-3). There is some controversy (4,5) as to the time course of their actions on thyroid gland tissue in vitro but both substances appear to exert their action on the thyroid by the adenyl cyclase stimulation (6).

LATS has been shown to be a gamma-globulin (7) (specifically an IgG) and can be produced by lymphocytes obtained from patients with Graves' disease and cultured under appropriate conditions (8,9).

Previous studies from this laboratory (10) demonstrated that purified LATS-IgG, like TSH, stimulates glucose metabolism and enhances lipolysis in guinea pig adipose tissue in vitro.

The studies reported here demonstrate that the lipolytic effect of LATS is delayed over that of TSH and that this may be due to a delay in binding of LATS by the adipose tissue. They also present suggestive evidence that both substances mediate their lipolytic responses via the adenyl cyclase activation and demonstrate that a lipolytic gamma-globulin (presumably LATS) is produced by Graves' disease lymphocytes in culture.

MATERIALS AND METHODS

In all experiments epididymal adipose tissue obtained from male albino guinea pigs (600-900 g) was used. The animals were

killed by a blow to the head under light ether anesthesia. Epididymal fat pads were excised from 2-4 animals and slices (50-100 mg) were randomly pooled in 0.9% NaCl solution. In some experiments slices totaling 200-300 mg were added to each 25 ml Erlenmeyer incubation flasks. In some experiments 0.2 ml of packed free fat cells prepared by the technique of Rodbell (11) were added to each flask. Incubations were for varying times (2 hours in most experiments) in Krebs-Ringer bicarbonate (KRB) containing 4% bovine serum albumin (BSA) and glucose 100 mg/100 ml. The gas phase was air and the incubations were carried out in a Dubnoff metabolic incubator at 37 C. At the end of incubation free fatty acids (FFA) were estimated by the method of Dole (12) on an aliquot of the incubation medium where slices were used or on an aliquot of the incubation medium plus the cells where free fat cells were used.

Thyrotropin was NIH-TSH-B4. Normal IgG and LATS-IgG were prepared in our own laboratory from various LATS containing sera by precipitation with 40% ammonium sulfate followed by DEAE-Sephadex chromatography. Results are expressed as micro-equivalents of free fatty acid per gram of wet tissue weight per hour in the case of tissue slices or as micro-equivalents of free fatty acids per flask per hour in the case of experiments utilising free fat cells. All groups were in triplicate.

BINDING EXPERIMENTS

In experiments where binding was measured tissue slices were either incubated in KRB containing 100 mU/ml of TSH or 3 mg/ml of LATS-IgG or were dipped for 5 sec in these solutions, washed in large quantities of 0.9% NaCl solution for 2 min and then incubated for 2 hr in KRB with no added test substances.

THEOPHYLLINE EXPERIMENTS

Dose response curves were obtained for theophylline, TSH and LATS-IgG. The amounts of the three tests substances which were just insufficient to cause significant enhancement of lipolysis was established. These were 0.2 mM theophylline, 10 μU/ml of TSH and 0.166 mg/ml of LATS-IgG. This ineffective amount of theophylline was added to the ineffective amounts of each of the test substances. A p value for interaction was estimated.

LYMPHOCYTE CULTURE EXPERIMENTS

Heparinized blood was obtained from a normal control and from a euthyroid patient with Graves' disease (previously treated with I^{131}). The Graves' disease patient had high levels of circulating LATS whereas the normal patient had no LATS detectable by the McKenzie mouse bioassay (13). Purified lymphocytes were prepared by standard methods and one aliquot

was cultured for 7 days in Eagle's medium containing 10% fetal calves serum plus phytohemagglutinin 0.05 ml/ml. The other aliquot of lymphocytes from each person was cultured in a similar manner but without the addition of phytohemagglutinin. At the end of 7 days only the cultures to which phytohemagglutinin had been added contained living cells.

At the end of the culture period the total culture bottle contents were centrifuged at 7,000 rpm for 20 min and the cells and sediment discarded. Half of the supernatant was precipitated with 40% ammonium sulfate and then redissolved to the original quantity in distilled water. This was then dialyzed against several changes of distilled water at 4 C for 2 days. In the lipolysis experiments 0.2 ml of the original supernatant or a similar quantity of the redissolved ammonium sulfate precipitate were added to each incubation flask.

RESULTS

In all experiments in which incubations were terminated at 15 min intervals after the commencement, the onset of the lipolytic action of LATS was delayed over that of TSH. Fig. 1 shows results of one experiment. It can be seen that although significant lipolysis was detectable with TSH 60 min after the commencement of the incubation, no significant effect of LATS could be seen until 90 min.

Binding of TSH by adipose tissue slices appears to occur very rapidly and is not reversible by simple washing. In Fig. 2 it can be seen that incubation of adipose tissue slices in KRB containing TSH 100 mU/ml caused a marked increase in lipolysis and that even contact of the slices with TSH for only 5 sec with subsequent rinsing and incubation in TSH free medium caused almost the same degree of enhancement of lipolysis. Incubation of the tissue slices in KRB containing LATS-IgG 3 mg/ml also caused marked enhancement of lipolysis. However, it can be seen that tissue dipped for a short while in LATS-IgG and incubated in LATS free medium showed no enhancement of lipolysis over the control normal human IgG.

Incubations with free fat cells in which sub-minimal amounts of both TSH and theophyline were added to the flask led to a marked lipolytic effect (Fig. 3). Similarly, incubations of free fat cells with sub-minimal amounts of LATS-IgG and theophyline also led to significant interaction. Although cyclic AMP levels or adenyl cyclase activity were not measured directly these theophyline experiments suggest that the actions of both of these substances are mediated via adenyl cyclase stimulation.

In Fig. 5 it can be seen that where phytohemagglutinin was absent there was no difference in the lipolytic effect of culture medium in which normal lymphocytes or Graves' disease

lymphocytes had been cultured. It is evident, however, that medium containing phytohemagglutinin in which Graves' disease lymphocytes had been cultured contained a lipolytic substance not present in normal lymphocyte medium. All of the lipolytic activity in the Graves' disease culture medium was contained in that fraction which precipitated with 40% ammonium sulfate (Fig. 6). These results demonstrate that Graves' disease lymphocytes cultured in the presence of a mitotic stimulant produce over a period of 7 days a lipolytic factor which precipitates like a gamma-globulin.

DISCUSSION

These studies show a delay in the lipolytic effect of LATS when compared to that of TSH. In studies involving effects of TSH and LATS on canine thyroid slices Field (4) also demonstrated a delayed in vitro effect of LATS compared to TSH. Burke (5) could demonstrate no time difference between TSH and LATS effects on sheep thyroid slices.

Pastan (14) has demonstrated rapid binding of TSH by thyroid slices. Our results demonstrate similar rapid binding of TSH by adipose tissue. There is little data concerning rapidity of LATS binding to thyroid tissue but data presented here shows it to be delayed over that of TSH. Whether the delay in binding of TSH by the adipose tissue slices is related to its later (than TSH) lipolytic action is not clear, but since

both appear to act by adenyl cyclase stimulation which involves as a first step binding to the receptor proteins this would be a logical conclusion.

The production of cyclic-AMP by adenyl cyclase appears to be a control site for many hormonal actions (15). There is good evidence that it is the mechanism of TSH (16) activation of thyroid tissue and Bastomsky and McKenzie (6) have produced evidence that it is also the mechanism by which LATS exerts its thyroid effects. Theophylline is a phosphodisterase inhibitor and thus retards the breakdown of cyclic-AMP. Significant interaction between a substance and theophylline is highly suggestive evidence that the substance mediates its action by adenyl cyclase stimulation. Our data suggest that both TSH and LATS mediate their lipolytic action on adipose tissue by this mechanism. That TSH stimulates adipose tissue cyclic-AMP production has been shown previously (17) but this is the first data suggesting a similar mechanism of LATS action on adipose tissue.

We have previously confirmed, by appropriate inhibition studies, that the lipolytic effect seen with LATS-IgG preparations is indeed due to its LATS content. (18). McKenzie (8) and later Miyai (9) demonstrated that the source of LATS in Graves' disease patients was circulating lymphocytes, however, Edmonds et al. (19)

were unable to confirm this. Our results demonstrate that Graves' disease lymphocytes in culture can produce a lipolytic gamma-globulin, although we have only demonstrated it in one case. Those data represents strong support for our belief that the lipolytic effect of LATS-IgG preparations is due to the LATS contained therein.

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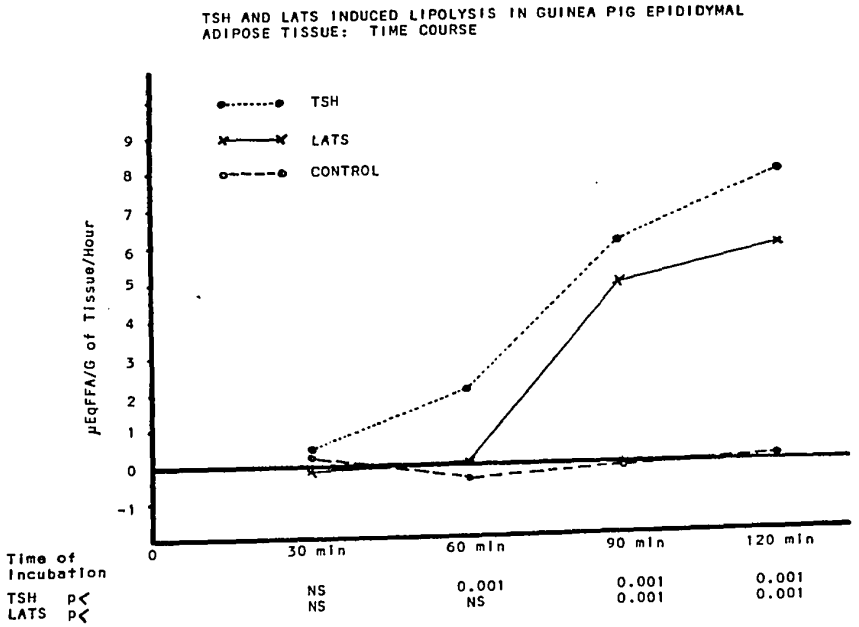
TSH AND LATS INDUCED LIPOLYSIS IN GUINEA PIG EPIDIDYMAL ADIPOSE TISSUE: TIME COURSE
TSH
LATS
CONTROL
μEqFFA/G of Tissue/Hour
9
8
7
6
5
4
3
2
1
0
-1
0
30 min
60 min
90 min
120 min
Time of Incubation
TSH p<
LATS p<
NS
NS
0.001
NS
0.001
0.001
0.001
0.001

Fig.1

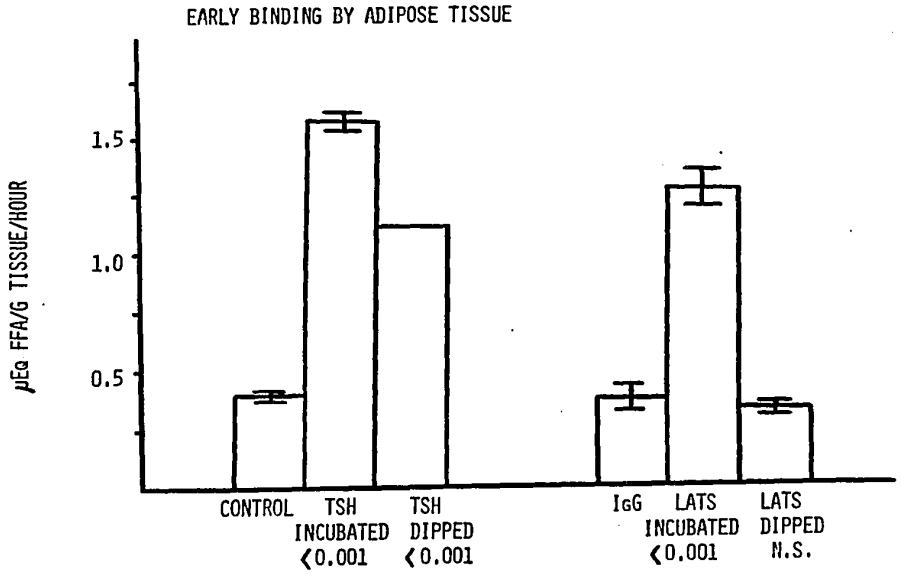
EARLY BINDING BY ADIPOSE TISSUE
μEq FFA/G TISSUE/HOUR
1.5
1.0
0.5
CONTROL
TSH INCUBATED
<0.001
TSH DIPPED
<0.001
IgG
LATS INCUBATED
<0.001
LATS DIPPED
N.S.

Fig.2

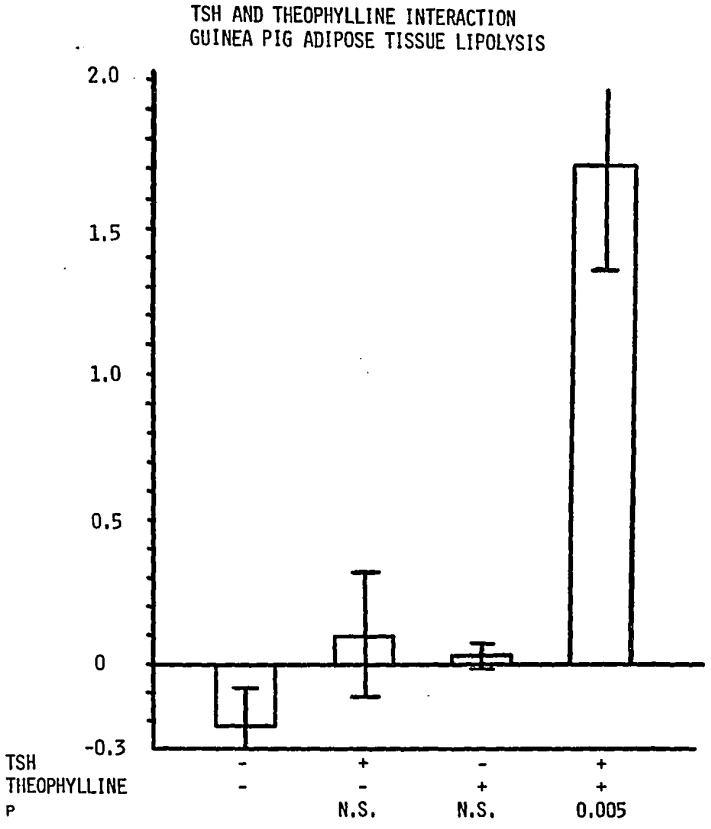
TSH AND THEOPHYLLINE INTERACTION
GUINEA PIG ADIPOSE TISSUE LIPOLYSIS
2.0
1.5
1.0
0.5
0
-0.3
TSH
THEOPHYLLINE
P
- + - +
- - + +
N.S. N.S. 0.005

Fig.3

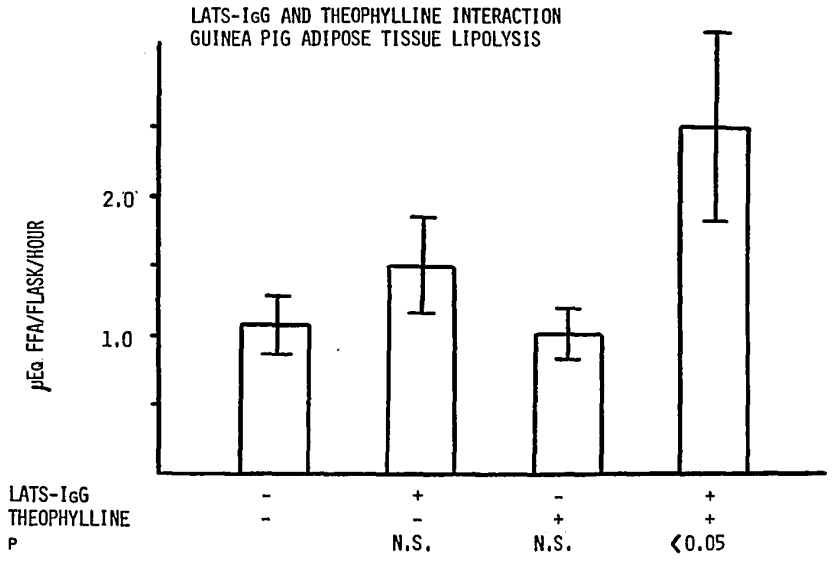
LATS-IgG AND THEOPHYLLINE INTERACTION
GUINEA PIG ADIPOSE TISSUE LIPOLYSIS
μEq FFA/FLASK/HOUR
2.0
1.0
LATS-IgG
THEOPHYLLINE
P
-
-
+
-
-
+
+
+
N.S.
N.S.
<0.05

Fig. 4

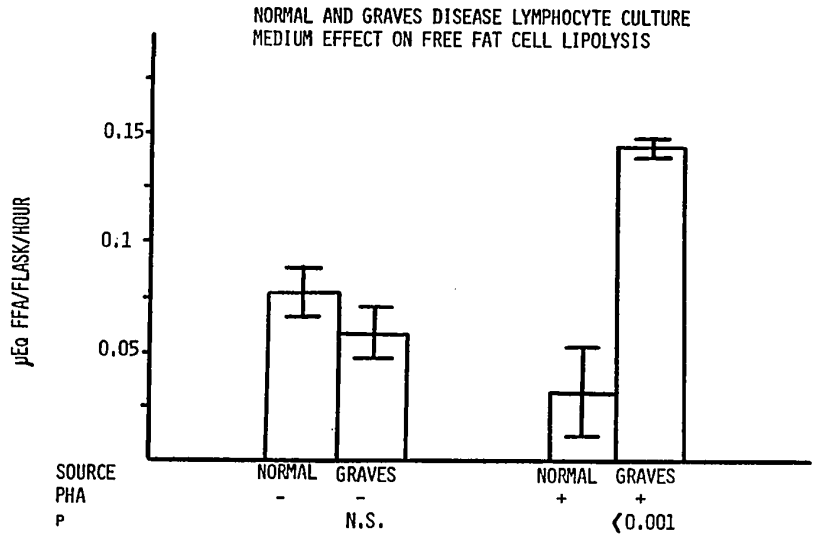
NORMAL AND GRAVES DISEASE LYMPHOCYTE CULTURE
MEDIUM EFFECT ON FREE FAT CELL LIPOLYSIS
µEq FFA/FLASK/HOUR
0.15
0.1
0.05
SOURCE
PHA
P
NORMAL
GRAVES
NORMAL
GRAVES
-
-
+
+
N.S.
<0.001

Fig.5

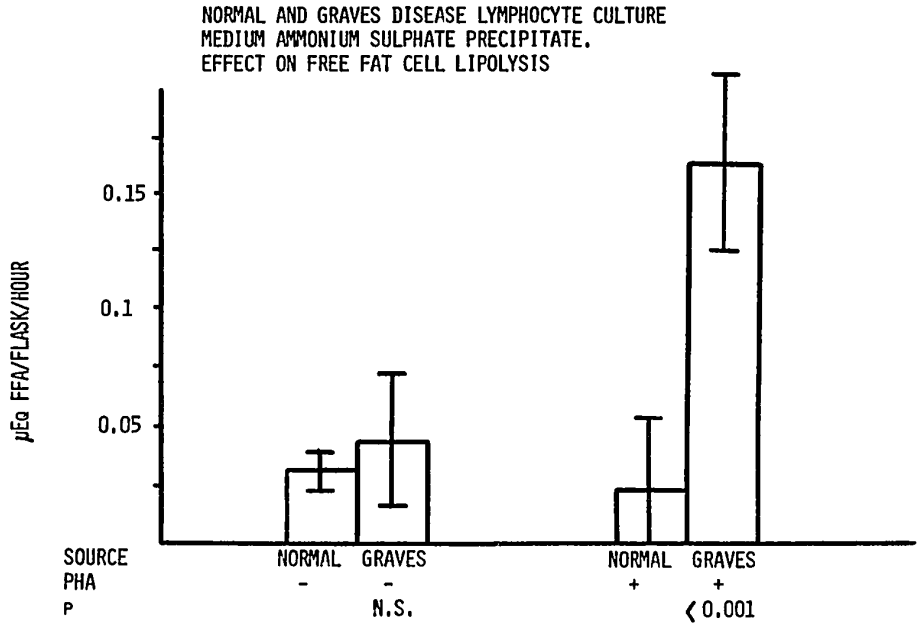
NORMAL AND GRAVES DISEASE LYMPHOCYTE CULTURE
MEDIUM AMMONIUM SULPHATE PRECIPITATE.
EFFECT ON FREE FAT CELL LIPOLYSIS
µEq FFA/FLASK/HOUR
0.15
0.1
0.05
SOURCE
PHA
P
NORMAL
GRAVES
NORMAL
GRAVES
-
-
+
+
N.S.
<0.001

Fig.6

The foregoing papers establish that LATS-IgG preparations are lipolytic in several species and characterise this action. We have shown that a lipolytic gamma-globulin is produced by Graves' disease lymphocytes in culture.

The next paper further confirms that this is indeed an effect of LATS that we are demonstrating by showing that manoeuvres which inhibit the mouse bioassay effect of LATS also inhibit the lipolytic effect. Also included is some early evidence that the lipolysis assay can detect LATS in unconcentrated serum from patients with Graves' disease.

THE LIPOLYTIC EFFECT OF LATS-IgG AND GRAVES' DISEASE SERA

BY

IAN R. HART

M.B., Ch.B., F.R.C.P.(C)

Since its discovery by ADAMS and PURVES (1956), the Long-Acting Thyroid Stimulator, LATS, has been shown to mimic many of the in vivo and in vitro effects of TSH on the thyroid (MCKENZIE, 1968; BURKE, 1968; KRISS, 1968). WHITE and ENGEL (1958), and later FREINKEL (1961), demonstrated that TSH had extrathyroidal effects in that it stimulated glucose oxidation and lipolysis in rat adipose tissue when added in vitro. We have recently demonstrated that LATS, like TSH, stimulates glucose metabolism in rat adipose tissue slices (HART and MCKENZIE, 1969). The studies reported here demonstrate that purified LATS-IgG enhances lipolysis in guinea pig adipose tissue slices and that this effect is also produced by unconcentrated sera containing LATS.

MATERIALS AND METHODS

Male guinea pigs (or in some experiments, rats or mice) were obtained from Quebec Breeding Farms. They were allowed food and water ad lib. Epididymal fat pads from 3-6 animals were pooled in 0.9% NaCl. Two to 4 pieces totalling 200-400 mgm were incubated in Krebs-Ringer Bicarbonate containing glucose 100 mgm/100 ml and 4% bovine serum albumin. Incubations (pH 7.4) were carried out at 37^{o}C with a gaseous phase of 95% O_2, 5% CO_2 and most incubations were for 2 hours. At the end of incubation 2 ml of incubation medium was removed and free fatty acids (FFA) were estimated by the method of

DOLE (1956). LATS assays were by the mouse bioassay (MCKENZIE and WILLIAMSON, 1966) and were performed in the laboratory of Dr. J. M. MCKENZIE.

TSH was NIH-TSH-B 4. Anti-TSH serum was prepared in rabbits by injection of 1 unit of TSH mixed with Freund's adjuvant at 5 day intervals for 5 doses. LATS-IgG was prepared from various LATS-containing sera by precipitation with 40% ammonium sulphate followed by DEAE Sephadex chromatography (KRISS et al., 1964). IgG was either commercially obtained or prepared in our laboratory from pooled normal sera as for LATS-IgG. Anti-IgG was horse antiserum to human IgG obtained commercially (Hyland).

Normal thyroid and liver microsomes were prepared by standard techniques from pooled fresh autopsy specimens.

RESULTS

TSH was markedly lipolytic when added to guinea pig adipose tissue in vitro (Fig. 1). Enhancement of lipolysis was produced by as little as 1 μU of TSH per ml and the effect was maximal with 1 mU/ml. Purified LATS-IgG also was markedly lipolytic in guinea pig adipose tissue and this effect was demonstrable with amounts of LATS-IgG which were not detectable in the mouse bioassay. Lipolysis was significantly enhanced by 0.166 mgm of LATS-IgG per ml (Fig. 2), an amount which injected per mouse in the bioassay gave a non-significant 9 hour response of 158%. Lipolysis was maximal with 0.66 mgm/ml, an amount

which gave only a 395% 9 hour response in the mouse bioassay.

Though we confirmed the well-known lipolytic effect of YSH in both rat and mouse adipose tissue, we were unable to demonstrate any lipolytic effect of LATS in these two species. Incubations with as much as 2.5 mgm/ml of LATS-IgG failed to demonstrate enhancement of lipolysis in both rat and mouse adipose tissues, even in incubations of up to 8 hours in duration. MUNRO et al. (unpublished observations) have been able to demonstrate a lipolytic effect of LATS-IgG on rat adipose tissue using a free fat cell preparation.

All purified LATS-IgG preparations so far tested have enhanced lipolysis in guinea pig adipose tissue. No lipolytic activity could be detected in any normal human IgG preparation (including that prepared in our own laboratory and those obtained from Sylvana, Protein Foundation, and Immunology Inc.).

The lipolytic effect of TSH and LATS-IgG could be inhibited by incubation with specific anti-sera (Fig. 3). The addition of 0.1 ml of rabbit anti-serum to TSH did not affect basal lipolysis or LATS-induced lipolysis, but completely inhibited TSH-induced lipolysis. Similarly, adding 0.2 ml of horse anti-serum to human IgG had no effect on basal lipolysis, or on TSH-induced lipolysis, but significantly reduced the lipolytic effect of LATS.

The specifity of the lipolysis effect of LATS-IgG was also demonstrated by the fact that it, like the thyroid-stimulating

effect in the mouse bioassay, could be inhibited by prior incubation with a thyroid microsome preparation (Fig. 4). Similar incubation overnight with simultaneously prepared liver microsomal preparation did not inhibit either the thyroid-stimulating or lipolytic effect of the LATS-IgG preparation.

The finding that purified LATS-IgG was lipolytic when added in vitro, and in quantities not detectable by bioassay, prompted us to look at the effect of unconcentrated sera from normal volunteers and that from patients with GRAVES disease on free fatty acid release from guinea pig adipose tissue. No lipolytic activity could be detected in any of the normal sera tested (Fig. 5). Normal human serum to which l-thyroxine had been added to bring the estimated total serum thyroxine level to 20 ug/100 ml also had no lipolytic effect (Fig. 5, SIN + Thyroxine). All 6 LATS positive sera from patients with GRAVES disease so far tested were markedly lipolytic (Fig. 6). Thirteen sera from patients with GRAVES disease in which no LATS was detected in the mouse bioassay, were tested for lipolysis. Four of these were found to be significantly lipolytic (Fig. 7).

DISCUSSION

These results indicate that LATS-IgG is lipolytic (at least in guinea pig adipose tissue) and in amounts undetectable in the mouse bioassay. This greater sensitivity may explain

the detection of lipolytic activity in the 4 sera from patients with GRAVES disease in which no LATS activity was detected by mouse bioassay. Little work has been done on the lipolytic effect of whole serum. One study (BURNS et al., 1967) reported that incubation of both rat adipose slices and rat free fat cells in normal fasting and fed human serum resulted in increased glyceride-glycerol release and glucose uptake. Obviously with such a complex solution as human serum which contains many potentially lipolytic agents, further studies are required on the specificity of the LATS lipolytic effect.

The significance of any lipolytic action of LATS in the human situation, i.e. in GRAVES disease, remains to be assessed. Abnormal elevations of FFA levels found in hyperthyroidism (RICH et al., 1959) appear to be related to the hyperthyroidism per se and are corrected when the patient is rendered euthyroid (VINIK et al., 1970). Also it has been reported that TSH has no lipolytic effect on human adipose tissue, either in vivo (RABEN, 1959) or in vitro (MOSINGER et al., 1965).

The preliminary results presented here however, suggest that enhancement of lipolysis in guinea pig adipose tissue slices may be a simple and sensitive method for detecting the presence of LATS in the serum, providing the specificity of this effect of whole LATS-containing serum can be further demonstrated.

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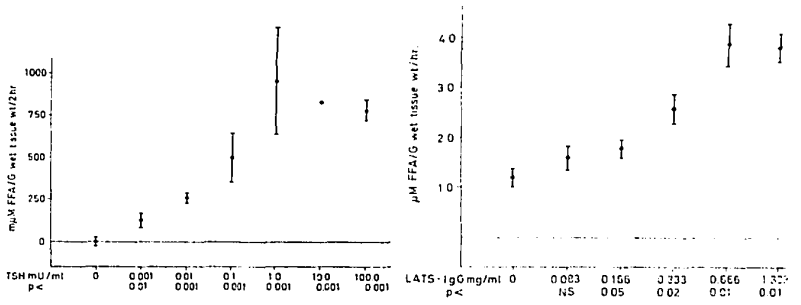


Fig. 1. Effect of TSH on lipolysis in guinea pig adipose tissue; dose-response relationship. The data are shown as the mean ± SD of triplicate observations.

Fig. 2. Effect of LATS-IgG on lipolysis in guinea pig adipose tissue; dose-response relationship. The data are shown as the mean ± SD of triplicate observations.

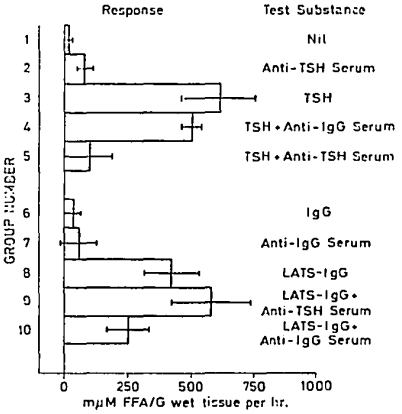
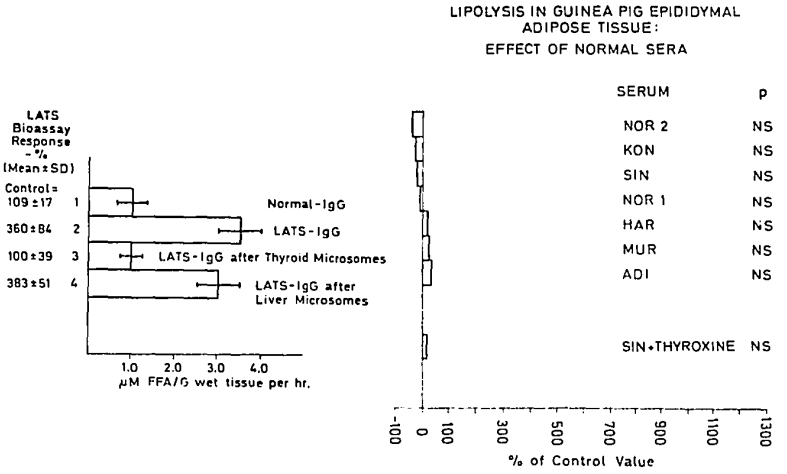


Fig. 3. Inhibition of TSH- and LATS-induced lipolysis in guinea pig adipose tissue by specific antisera to TSH was tested at 100 mU/ml, normal- and LATS-IgG at 3.5 mgm/ml, anti-TSH serum at 0.1 ml/flask and anti-IgG serum at 0.2 ml/flask.

p values for significance of differences of means are as follows:

Groups compared	p	Groups compared	p
1 vs 2	ns	6 vs 7	ns
1 vs 3	0.001	6 vs 8	0.001
3 vs 4	ns	8 vs 9	ns
3 vs 5	0.001	8 vs 10	0.05
4 vs 5	0.001	9 vs 10	0.01



▲

Fig. 4. Effect of pre-incubation with Thyroid and Liver microsomes on the lipolytic activity of LATS-IgG. The data are shown as the mean ± SD of triplicate observations. P values for significance of differences of means are as follows:

Groups compared	p	Groups compared	p
1 vs 2	0.001	2 vs 3	0.001
2 vs 4	ns		

LATS-activity in preparations 2, 3 and 4 was assayed in mice and the responses are listed as % increases in blood radioactivity (mean ± SD); control solution was 1% human serum albumin in 0.9% NaCl.

▲

Fig. 5. Lipolysis in guinea pig adipose tissue: Effect of normal human sera.
Amount added per flask: NOR 1 and NOR 2 0.5 ml
All others 0.4 ml

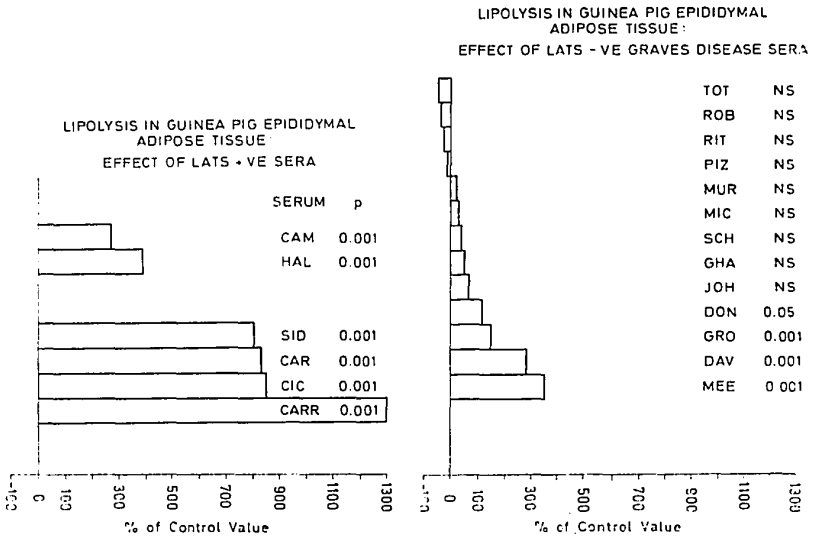


Fig. 6. Lipolysis in guinea pig adipose tissue: Effects of LATS positive sera.
Amount added per flask: CAM and HAL 0.2 ml
Others 0.4 ml

Fig. 7. Lipolysis in guinea pig adipose tissue: Effect of LATS negative GRAVES' Disease sera.
Amount added per flask: 0.2 ml

Finally a practical importance for the LATS-IgG lipolytic effect is presented. LATS can be detected in unconcentrated sera from patients with Graves' disease by its lipolytic effect more frequently than it is detected by the mouse bioassay.

The fact that data is presented showing that by this method LATS can be detected in appropriately treated sera of 91% of cases of Graves' disease, re-establishes a central role for this substance in that condition.

SERUM LIPOLYTIC FACTORS IN DYSTHYROID STATES

BY

IAN R. HART

M.B., Ch.B., F.R.C.P.(C)

That purified LATS-IgG has a lipolytic effect when added to adipose tissue in vitro was first demonstrated by Hart and McKenzie in 1969 (1). This action has since been confirmed using a different species (2). Later work from this laboratory demonstrated that a lipolytic effect of purified LATS-IgG was also detectable on human adipose tissue free fat cells in vitro (3).

We have previously demonstrated (3) that bovine TSH has a lipolytic effect on human adipose tissue in vitro confirming the previous work of Bray (4). In these studies it was apparent that human adipose tissue was much more sensitive to human TSH than to bovine TSH.

The current studies demonstrate that both TSH and LATS can be detected in some sera by their lipolytic effect and further show that the lipolytic assay for LATS is more sensitive than the currently used McKenzie mouse bioassay (5). By the lipolytic technique LATS is detectable in IgG concentrates from the sera of up to 91% of patients with Graves' disease.

MATERIALS AND METHODS

In all experiments the tissue used was guinea pig epididymal adipose tissue slices. Albino guinea pigs (600-900 g) were maintained at constant temperature and allowed food and water ad lib. for at least 10 days prior to sacrifice. The animals were killed by a blow to the head under light ether anesthesia.

Epididymal fat pads were removed and cut into slices (50-100 mg each). The tissue slices were pooled in 0.9% NaCl solution. Two to four slices totaling 150-300 mg of tissue were added to each 25 ml Erlenmeyer flask. Incubations were in Krebs-Ringer bicarbonate (KRB) containing glucose 100 mg/100ml and 4% bovine serum albumin (BSA). Incubations were at pH 7.4 and temperature 37 C for 2 hr. All groups were in triplicate. Control or test substances $\pm$ specific antisera were added at the beginning of incubation. Lipolysis was detected by the release into the medium of either free fatty acids (FFA) estimated by the Dole method (6) or glyceride-glycerol measured by the Wieland method(7).

SERA

In experiments where sera were being tested 0.2 ml of serum was added to each flask. Several pools of normal sera were used as controls. None of these pooled normal sera were lipolytic. Fifty sera from patients with no known thyroid disease were tested. Serum was obtained from 86 documented cases of Graves' disease. Sera of 10 patients with clinical and laboratory documented hypothyroidism were tested. Twenty-two sera from patients with miscellaneous thyroid conditions were also tested including 10 euthyroid patients with nodular goitre and 8 with Hashimoto's thyroiditis. All patients in the above groups had PBI, T_4C, T_4-BP, T_3-Uptake and I^{131} Uptake determined.

ANTISERA

Antiserum to NIH-TSH-B4 was prepared in rabbits by injection of 1 u of TSH mixed with Freund's adjuvant at 5 day intervals for 5 doses. Antiserum to human IgG was a horse antiserum obtained commercially (Hyland).

LATS

All LATS measurements were performed by the McKenzie mouse bioassay method (5) and were carried out in the laboratory of Dr. J. M. McKenzie in Montreal. The sera of 32 patients with Graves' disease was precipitated with 40% ammonium sulfate followed by DEAE-Sephadex chromatography (8). The equivalent weight of purified IgG from 1 ml of serum was added to each test flask. Thus, compared to the serum studies where 0.2 ml was added to each flask there was a fivefold concentration of IgG in these experiments.

RESULTS

Whereas only 6% of normal sera caused enhancement of lipolysis, 50% of Graves' disease and 70% of hypothyroid sera were significantly lipolytic when added to this system (Fig.1 and 2). None of the sera obtained from patients with other thyroid conditions were lipolytic.

There was no correlation between the presence or absence of antibodies to thyroglobulin or to the level of thyroglobulin

antibody titre to the lipolytic activity of a serum (Fig. 3). Since both thyroxine and triiodothyronine are known to influence adipocyte metabolism of some species including man (9) it was important to rule out that the lipolytic effect of the Graves' disease sera was due to high levels of circulating thyroid hormones. Fifty of the Graves' disease patients had been part of another study in which the thyroid status had been definitively ascertained by expert clinical evaluation combined with 7 thyroid function tests. As shown in Fig. 4 hyperthyroidism did not lead to higher instance of lipolytic sera. Though the number of hypothyroid Graves' disease sera is small appears to be an increased chance of the serum being lipolytic if the patient is also hypothyroid.

Of the 86 Graves' disease sera tested for lipolysis, 40 were also tested for LATS in the mouse bioassay. Only 35% of these gave positive LATS responses (Fig. 5). All of the sera which were positive for LATS in mouse bioassay were also positive in the lipolysis assay (Fig. 6).

Although only 50% of unconcentrated sera from patients with Graves' disease were lipolytic, when fivefold IgG concentrates from 32 of these sera were tested, 29 (91%) caused enhancement of lipolysis in the system (Fig. 7).

Seven Graves' disease sera were examined regarding the effect of the addition of antiserum to IgG and 5 of these were

also tested with antiserum to TSH. As shown in Fig. 8 addition of 0.2 ml of IgG antiserum produced significant inhibition of the lipolytic activity of all samples tested. Four patients who had primary hypothyroidism and whose sera were lipolytic were examined in a similar manner. All 4 sera tested significantly inhibited by the addition of antiserum to TSH but 2 of the sera were also partially inhibited by the addition of antiserum to IgG.

DISCUSSION

The data presented raises three main issues -

1. The in vitro lipolytic assay is more sensitive than the mouse bioassay.
2. LATS is detectable by this method in almost all cases of Graves' disease suggesting a central pathogenetic role for this substance.

and 3. Since both LATS and high levels of TSH are detectable in appropriate sera by this method and since both substances affect human adipose tissue (3), what role do these substances play in the lipid abnormalities occurring in Graves' disease and hypothyroidism?

Since the earliest modification of Adams LATS assay (10) by McKenzie (11) in 1958, the mouse bioassay technique has become the world-wide standard for detecting and measuring LATS in serum. The mouse bioassay is difficult to establish, expensive with regards to personnel and animal upkeep and involves use of radioactive materials. It has often been accused of insensitivity (12-14) and has often had the problem of non-specific responses (15). The problems associated with the assumption of single effects on one organ of substances injected into the bloodstream of an animal are clearly demonstrated by the experience of finding that the apparent LATS effect of the protein produced by immunization of animals with human thyroid tissue, actually was due to a thyroxine binding effect (16).

The lipolytic assay presented in previous papers and standardised here is more sensitive than the mouse bioassay and since it is performed in vitro eliminates the problem of whole animal effects. Since many substances present in serum are potentially lipolytic the problem of specificity can be overcome by demonstrating inhibition of any lipolytic response due to LATS by antiserum to IgG. The assay is relatively simple and inexpensive to perform and could be carried out in any hospital or institution with small animal facilities and the ability to measure FFA or glyceride-glycerol. Since we have demonstrated that all mouse bioassay positive LATS sera are also lipolytic at very least the lipolytic assay might be used as a screening test for LATS.

Since its discovery in 1956 (10), the role of LATS in producing the cardinal triad of Graves' disease (hyperthyroidism, ophthalmopathy and dermopathy) has never been well defined. Following early enthusiasm that it explained all of the manifestations of Graves' disease LATS has progressively come to be closely associated with only the pretibial myxoedema and the hyperthyroidism. Recently its role in the production of the hyperthyroidism has been questioned by several authors although McKenzie (17) had disputed this view. One of the major arguments of those opposed to the assignation of a central role of LATS in the pathogenesis of the Graves' disease is the inability

to detect LATS in the serum of most patients with Graves' disease. It is detectable by the mouse bioassay in the unconcentrated serum of 10-45% of such patients and in only 70% even with tenfold serum concentration to IgG (18, 19). The data presented in this paper support a central role for LATS in Graves' disease, in that with fivefold serum concentration the stimulator is detectable in 91% of patients with this condition by the lipolytic assay. That the lipolytic activity of Graves' disease sera is not due to another antithyroid IgG (e.g. antibody to thyroglobulin) or to hyperthyroidism per se is shown by the lack of correlation with the presence of tanned red cell antibody or the patients' thyroid status.

The lipid abnormalities occurring in Graves' disease and hypothyroidism are not well defined. It would appear that the high circulating levels of FFA and glyceride-glycerol known to occur in hyperthyroidism revert to normal as the patient becomes euthyroid (20), but certainly acute adrenergic blockade does not achieve this effect (21). The unexplained low post-heparin lipolytic activity found in Graves' disease does not revert to normal with treatment of the hyperthyroidism (23) and the relationship of this lipase activity to LATS has not been fully examined. Although decreased basal lipolysis and decreased sensitivity

to catecholamine-induced lipolysis is evident in hypothyroid tissue (9) plasma concentration of FFA are often normal in hypothyroidism. The role of high circulating levels of TSH - high enough to be detectable by its lipolytic action on guinea pig adipose tissue in vitro as demonstrated in our studies - in maintaining normal fasting FFA levels in hypothyroidism remains to be elucidated.

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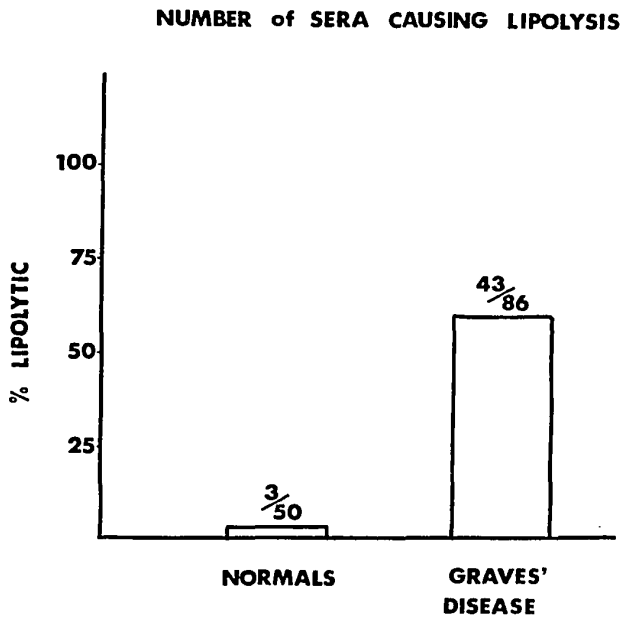
NUMBER of SERA CAUSING LIPOLYSIS
% LIPOLYTIC
100
75
50
25
43/86
3/50
NORMALS
GRAVES' DISEASE

Fig.1

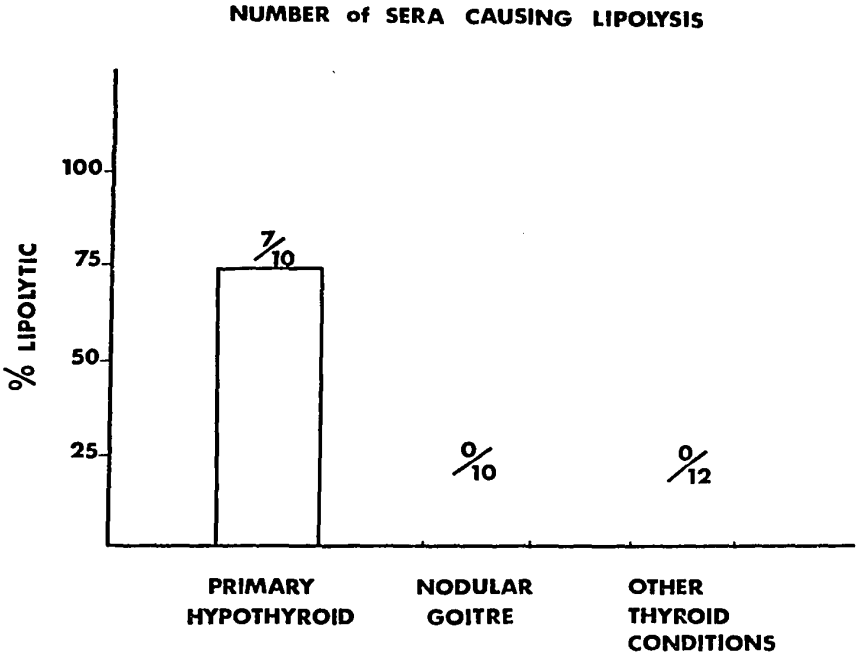
NUMBER of SERA CAUSING LIPOLYSIS
% LIPOLYTIC
100
75
50
25
7/10
0/10
0/12
PRIMARY HYPOTHYROID
NODULAR GOITRE
OTHER THYROID CONDITIONS

Fig. 2

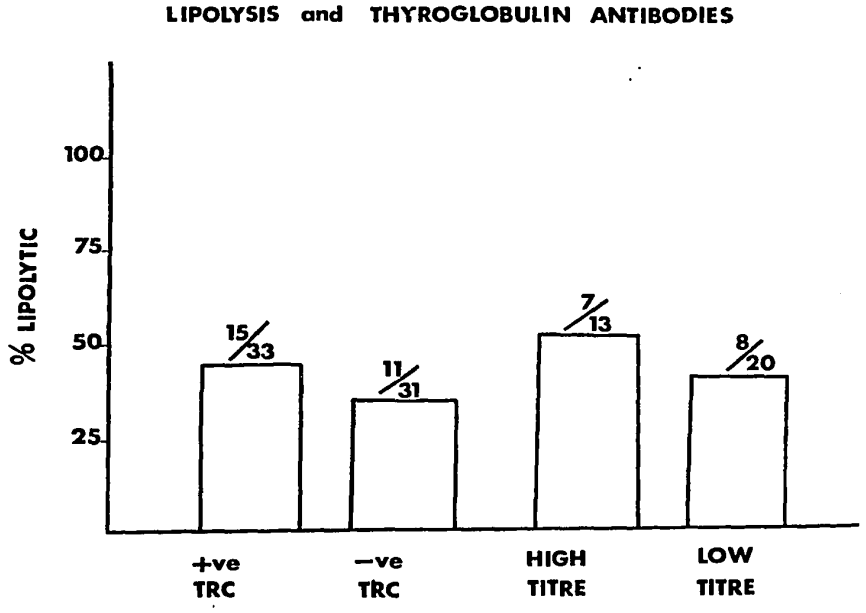
LIPOLYSIS and THYROGLOBULIN ANTIBODIES
% LIPOLYTIC
100
75
50
25
15/33
11/31
7/13
8/20
+ve TRC
−ve TRC
HIGH TITRE
LOW TITRE

Fig.3

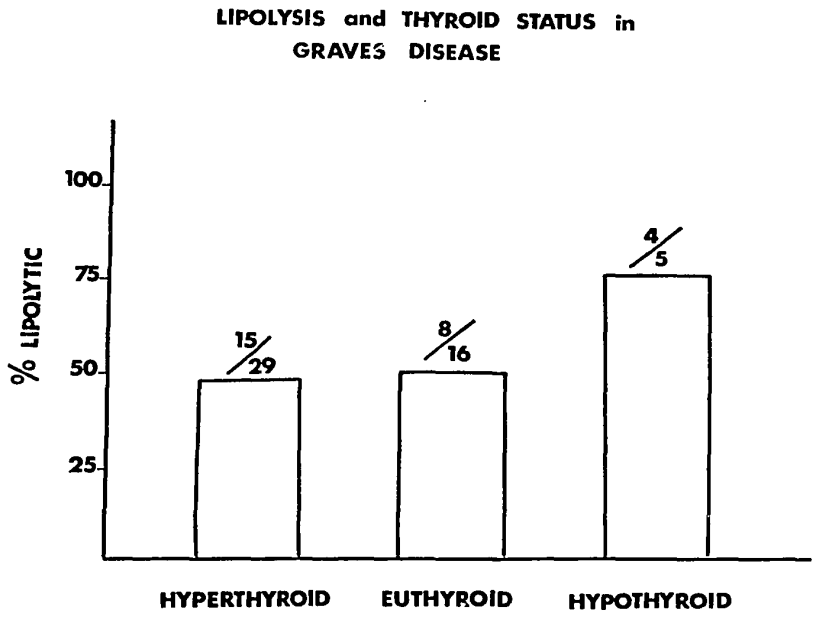
LIPOLYSIS and THYROID STATUS in
GRAVES DISEASE
% LIPOLYTIC
100
75
50
25
15/29
8/16
4/5
HYPERTHYROID
EUTHYROID
HYPOTHYROID

Fig.4

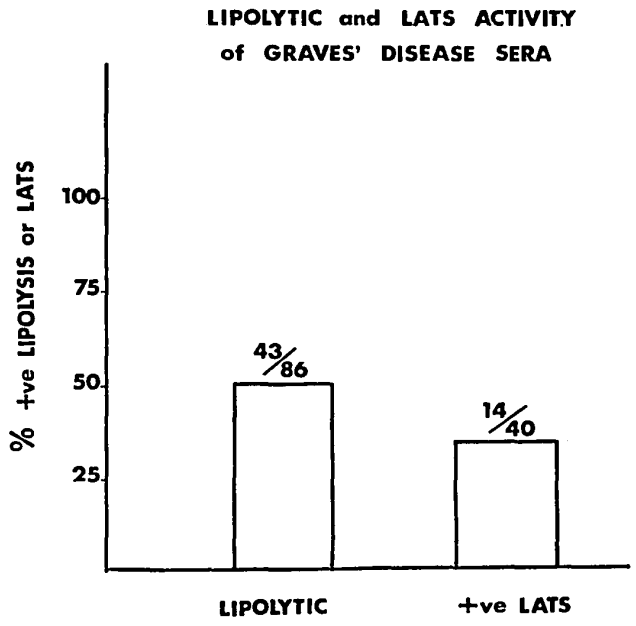
LIPOLYTIC and LATS ACTIVITY
of GRAVES' DISEASE SERA
% +ve LIPOLYSIS or LATS
100
75
50
25
43/86
14/40
LIPOLYTIC
+ve LATS

Fig. 5

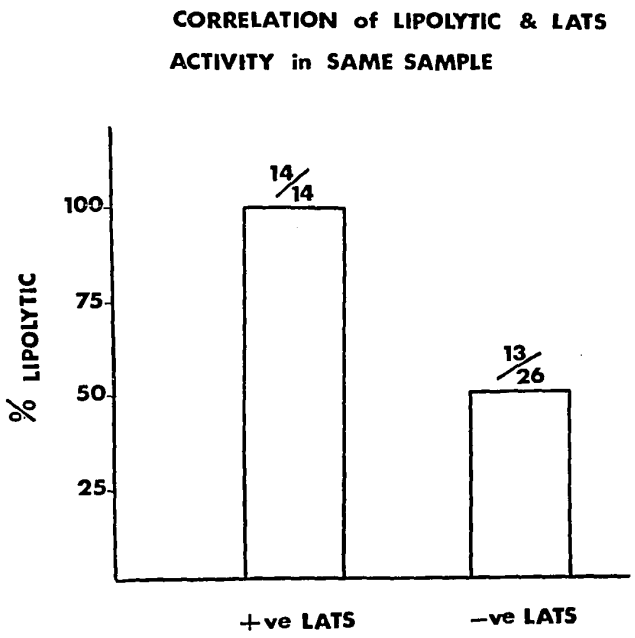
CORRELATION of LIPOLYTIC & LATS
ACTIVITY in SAME SAMPLE
% LIPOLYTIC
100
75
50
25
14/14
13/26
+ve LATS
−ve LATS

Fig.6

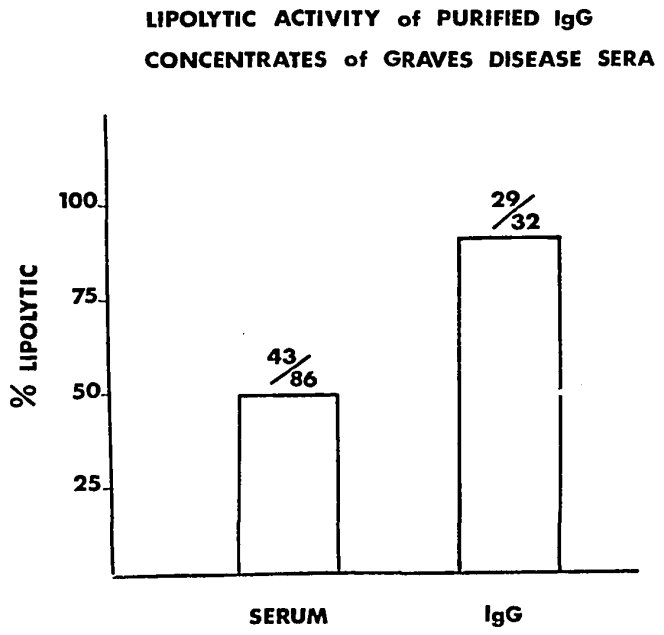
LIPOLYTIC ACTIVITY of PURIFIED IgG
CONCENTRATES of GRAVES DISEASE SERA
% LIPOLYTIC
100
75
50
25
43/86
29/32
SERUM
IgG

Fig.7

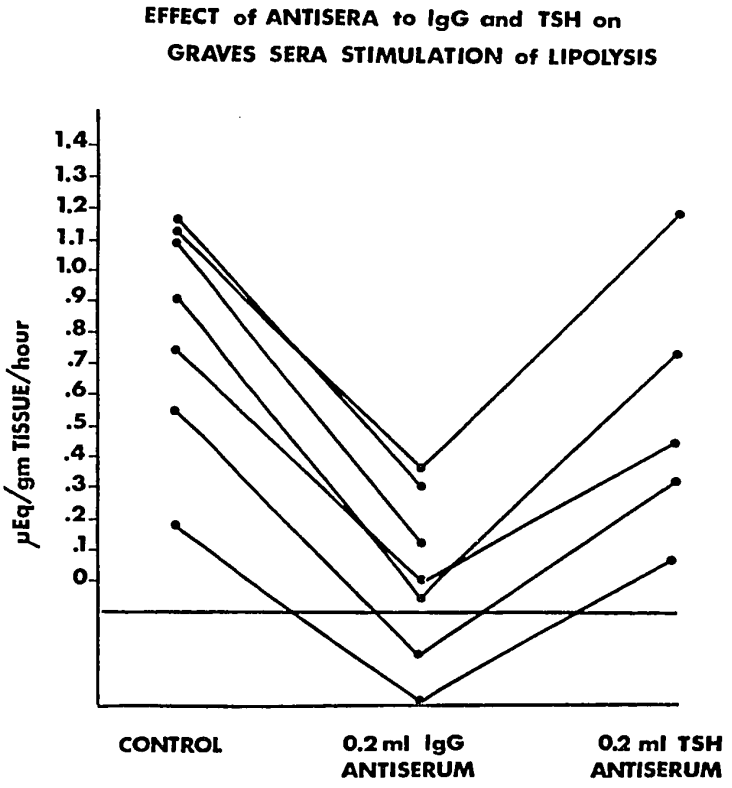
EFFECT of ANTISERA to IgG and TSH on
GRAVES SERA STIMULATION of LIPOLYSIS
μEq/gm TISSUE/hour
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
CONTROL
0.2 ml IgG ANTISERUM
0.2 ml TSH ANTISERUM

Fig.8

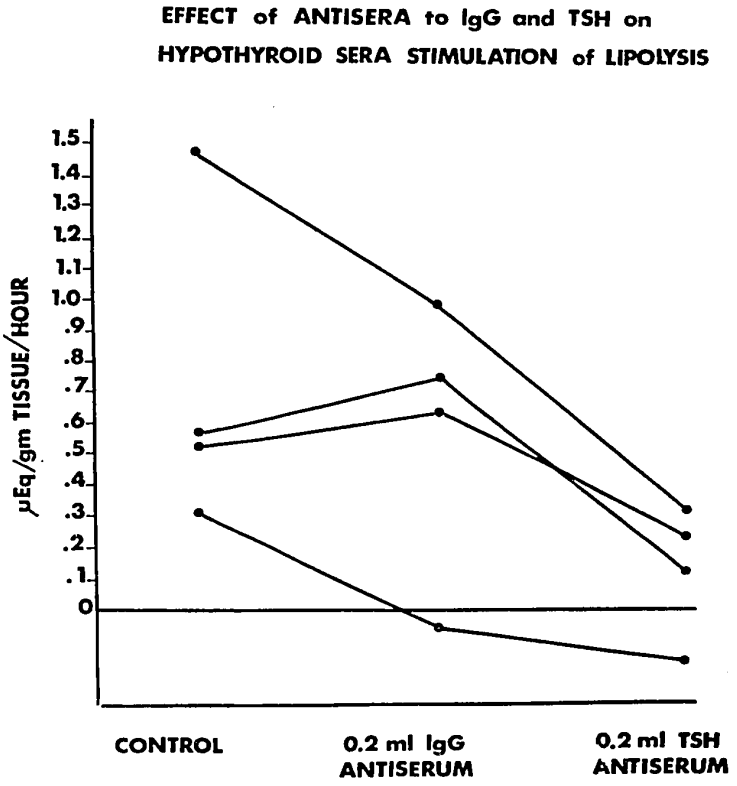
EFFECT of ANTISERA to IgG and TSH on
HYPOTHYROID SERA STIMULATION of LIPOLYSIS
μEq/gm TISSUE/HOUR
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
CONTROL
0.2 ml IgG ANTISERUM
0.2 ml TSH ANTISERUM

Fig.9

SUMMARY - CONCLUSIONS

The results presented in the foregoing five papers demonstrate that -

1. TSH and LATS have similar actions on glucose metabolism in guinea pig adipose tissue slices.

2. TSH is lipolytic when added to adipose tissue in vitro

   a) This was demonstrable in all 4 species tested - guinea pig, mouse, rat and human - in decreasing order of sensitivity.

   b) Human adipose tissue was more sensitive to human TSH than to bovine TSH.

   c) The TSH lipolytic effect was inhibited by the addition of specific antiserum.

   d) TSH was rapidly bound to adipose tissue.

   e) Indirect evidence (i.e. enhancement by theophyline) suggest that the TSH lipolytic effect is mediated via adenyl cyclase.

   f) Adipose tissue from various sites in the guinea pig are sensitive to TSH but the epididymal site is the most sensitive.

3. Purified LATS-IgG is lipolytic when added to the adipose tissue of some species in vitro.

   a) Guinea pig and rat (free fat cell preparations) adipose tissue are more sensitive than that of the human, whereas no effect is detectable on that of the mouse.

   b) Using guinea pig adipose tissue the lipolytic effect is a more sensitive detector of the presence of LATS than the mouse bioassay.

   c) The lipolytic effect of LATS in vitro is delayed over that of TSH.

   d) Unlike TSH, LATS is not rapidly bound to adipose tissue.

   e) The enhancement of lipolysis by LATS is adenyl cyclose mediated.

The evidence that the lipolytic effects of these IgG preparations is due to the LATS content and not simply a non-specific IgG effect includes our demonstration that -

   a) A lipolytic effect could not be detected using comparable amounts of several purified IgG preparations from pooled sera and from different sources.

b) The LATS-IgG induced lipolysis was consistently inhibited by the addition of antisera to human IgG but not antisera to TSH.

c) The LATS-IgG lipolytic effect was inhibited by pre-incubation with thyroid microsomes but not by liver microsomes.

d) A lipolytic gamma-globulin is produced by cultured lymphocytes from a patient with Graves' disease but not those from a normal person.

4. Both TSH and LATS can be detected in some unconcentrated sera by their lipolytic action.

a) The sera from 70% of hypothyroid patients is lipolytic and the lipolytic effect can be inhibited by the addition of antisera to TSH.

b) The sera from 50% of patients with Graves' disease is lipolytic and this effect is not related to the presence or absence of thyroid antibodies or the patients' thyroid status.

c) Where tested the lipolytic effect of the Graves' disease sera was inhibited by antisera to human IgG.

d) This lipolytic effect of Graves' disease sera is a more sensitive indicator of the presence of LATS than the mouse bioassay.

e) Fivefold concentration to IgG of Graves' disease sera demonstrates that LATS is detectable (by its lipolytic effect) in 91% of cases.

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