The long-acting thyroid stimulator

When injected into suitably prepared guinea pigs or mice, the serum of some patients with thyrotoxicosis causes a prolonged discharge of radioiodine from the thyroid. There is chemical and immunological evidence that the long-acting thyroid stimulator (LATS) responsible for this effect is distinct from pituitary thyroid-stimulating hormone, and that the activity is inherent in specific immunoglobulin G molecules. The distribution of thyroid-stimulating activity in the polypeptide chains of the IgG parallels antigen-binding activity in known antibodies. As a result of these findings and the specific absorption of LATS by human thyroid endoplasmic reticulum, it has been suggested that LATS is an autoantibody to a thyroid component. This hypothesis is discussed. Using suitable concentration methods LATS can be detected in the serum of 85 per cent of thyrotoxic patients, and the level correlates with several parameters of thyroid activity. High levels of LATS are also frequently associated with localized myxedema, but the relation of LATS to the eye signs associated with thyrotoxicosis is less clear.

Keith J. Dorrington, B.Sc., Ph.D.,* and Donald S. Munro, M.D. Sheffield, England
Department of Pharmacology and Therapeutics, University of Sheffield

The long-acting thyroid stimulator (LATS) was discovered by Adams and Purves in the serum of a patient suffering from thyrotoxicosis. Adams and Purves were using their sensitive bioassay for thyroid-stimulating hormone (TSH) for measurements in serum samples. This bioassay method followed the release of radioiodine from the thyroid glands of guinea pigs treated with thyroid hormone and was the basis of the later, and now more widely used, procedure of McKenzie. The discovery of LATS was made by a chance observation that the blood radioiodine of guinea pigs, which had been injected the previous day with serum from a patient with thyrotoxicosis, was greater than in other animals injected with control solutions. It had earlier been demonstrated in the same laboratory that there was occasionally a material in the circulation of patients with thyrotoxicosis which was capable of causing the histological changes associated with stimulation of the guinea pig thyroid. The histological demonstration of thyroid-stimulating activity was manifestly much less sensitive than the bioassay of Adams and Purves, and no doubt the first hypothesis being tested by these workers was that thyroid stimulation in thyrotoxicosis resulted from an excess of circulating TSH. By their astute observation of the delayed time of the peak effect of thyroid stimulation in their measurements of radioiodine discharge, Adams and

*Present address: Department of Biochemistry, Duke University Medical Center, Durham, N. C.
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Purves discovered the outstanding difference between the time courses of action of TSH on the one hand and of LATS on the other (Fig. 1). Although the precise time course differed slightly when such experiments were transferred to the white Swiss mice used in the McKenzie bioassay, the delayed time of maximum effect of LATS remains the chief characteristic by which it is differentiated from TSH.

Assay method

The method of McKenzie has been criticized in recent publications for its relative lack of precision, but there can be little doubt that it is the most widely used bioassay for TSH and LATS today. The more elaborate assay designs which have been described in different centers have not yet been in use long enough to clarify the advantages which their originators have claimed for them. Without actually altering McKenzie’s assay design, Kriss, Pleshakov, and Chien have greatly simplified the detection of LATS by using the intraperitoneal route for the serum injection and 16 hours for the critical blood sample. It is probable that many workers will take advantage of this simplified procedure and with the use of the intraperitoneal route may lessen the incidence of severe toxic effects, which are occasionally seen after injection of human serum into mice. Scrutiny of the numerous reports from different centers which have used the McKenzie bioassay reveals many minor differences in the strain of mouse used, the dose of radioactive iodine administered, the isotope of iodine selected, the method used for suppression of endogenous TSH secretion, the method of obtaining venous blood samples, and the time of blood sampling from the mice. There is also considerable variation in the methods used for the preparation of serum for intravenous injection. Some workers have assayed samples as soon as practicable; others have preferred a period of storage at –20°C. To minimize toxic reactions from serum injections, preliminary dialysis with isotonic solutions or gel filtration on Sephadex G-25 has been used.

In spite of these variations there has been a remarkable measure of agreement between different centers so far as the sensitivity to bovine TSH and the detection of high levels of LATS are concerned. It is important to note the way in which authors record the magnitude of their assay responses. In general, three different methods have been used: (1) giving the absolute response obtained in the assay, which is our practice, (2) deducting 100 per cent from this figure, and (3) deducting the mean effect of a control injection. The preferred method is usually indicated, so that no insuperable difficulty arises in relating the responses reported in different papers. What remains more controversial is the significance of smaller increases in blood radioactivity at the time of peak effect of LATS. Adams has indicated that some of these responses may be nonspecific effects of serum injections in the assay. This point is of sufficient importance to warrant discussion under a separate heading.

Nonspecific effects in the McKenzie bioassay. It is not by any means universally
accepted that nonspecific effects occur in all laboratories. Because of the differences in the details of the preparation at different centers, it is clearly impossible for any one group to do more than state what their own experience has been. The problem is closely related to the choice of an inert solution for control injections and most reports have tended to substitute solutions which contain protein or some other osmotically active component in the hope that the physical effects of a comparable volume of serum or extract of high protein content would be mimicked. In earlier papers several supposedly inert control solutions were used including isotonic sodium chloride, buffered salt solutions with varied concentrations of bovine or human albumen, and dextran-saline mixtures. Adams has been primarily responsible for pointing out that none of the control solutions which had been used were necessarily without any effect on radioiodine discharge in the assay animals. Although this point may, at first sight, seem to be of little importance, the significance of low levels of response in the assay depends upon the stand taken by different authors. There are those who rely entirely upon applying Student's t test for assessing differences between the effects of serum or a serum extract and the chosen control solution. Others consider that this criterion is not adequate because in their hands control solutions have caused a sporadic but sometimes significant release of $\text{I}^{131}$ in occasional batches of mice. For example, Dorrington has shown that a statistically significant difference can occur between the effects of saline and dextran-saline discharge. Serum can also cause nonspecific responses; and concentration procedures of proved efficacy for LATS may give disappointingly low yields when applied to serum which initially caused responses below 300 per cent in the assay yet achieved statistical significance when compared with the effects of an inert control injection. It is, therefore, the authors' opinion that there is need for great caution in interpreting all serum responses below 300 per cent, but it is not clear whether this reservation does or does not apply to materials other than serum. It would seem prudent to assume that it does. Clearly, low responses can also result from low levels of LATS, but to confirm its presence concentration procedures are always desirable.

Evidence that LATS is a true stimulator of thyroid function

With the type of assay preparation used to detect LATS, it is clear that anything which damaged thyroid cells might interfere with the continued retention of radioactivity in the gland and thus cause an increase in blood radioiodine. Many workers have stressed that injections of human serum, especially when fresh, were toxic to mice. The toxic effects of serum vary greatly in severity but death may occur after the administration of 0.5 ml., intravenously, although this is rare. Dialysis and gel filtration are both effective in eliminating the toxic effects of human serum which are, therefore, presumably due to substances of relatively low molecular weight. Dialysis does not diminish the LATS level of serum. It is thus unlikely that LATS is related in any way to these toxic factors, but it is not clear what relationship such influences may bear to the nonspecific responses caused by some samples.

Moreover, it has been clearly demonstrated that serum containing LATS can stimulate several other parameters of thyroid function as well as discharge of radioiodine. For example, LATS increased the thyroid uptake of $\text{I}^{131}$ and produced the histological changes associated with increased thyroid activity. Injection of LATS also increased the chemical protein bound iodine of experimental animals and stimulated the uptake of tritiated thymidine by the nuclei of thyroid cells. It is now accepted that a thyrotrophin releasing factor (TRF) is elaborated in the hypothalamus and acts on the pituitary

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*Crane, Major, and Munro: Unpublished observations.
to regulate the release of TSH.\textsuperscript{86} It was clearly necessary to consider whether LATS and TRF were related. However, in experiments on hypophysectomized mice, LATS was fully effective in the absence of the pituitary\textsuperscript{10, 74} indicating that it acted directly on the thyroid gland and that TRF was not involved.

**Differences between LATS and TSH**

In early experiments it became clear that there were significant differences in the chemical and biological properties of the two thyroid stimulators quite apart from their distinctive time courses of action in the bioassay.

**Antigenic differences.** Failure to neutralize LATS activity with antisera to human or bovine TSH under conditions which completely inactivated the homologous antigen established that LATS and TSH were antigenically distinct.\textsuperscript{7, 63} Werner and co-workers\textsuperscript{98, 101} however, had previously reported neutralization of LATS by an antiserum to bovine TSH. In their first paper the assay observations were not timed to detect both stimulators and the concentration method used is now known to destroy LATS. The second paper showed that a concentrate of the antibodies made from an antiserum to TSH neutralized LATS, This may have been due to contamination of the original TSH used as antigen with IgG; LATS is known to be associated with IgG and is neutralized by anti-IgG.

Darrington and Munro\textsuperscript{30} approached this problem by using antisera to TSH and LATS. Each antiserum was only effective against the homologous antigen and no cross-reaction could be demonstrated between LATS and TSH. Other evidence on the immunological distinction between TSH, LATS, and its chemically produced fragments is reviewed in a later section.

**Chemical and physical differences.** The alcoholic-saline extraction method of Bates, Garrison, and Howard\textsuperscript{13} destroyed LATS in serum\textsuperscript{25, 84} although it was effective in extracting TSH. In serum, TSH has been shown to be more susceptible than LATS to the action of the proteolytic enzymes pancreatin, trypsin, and chymotrypsin.\textsuperscript{59} However, TSH appears to be more resistant to heat inactivation than LATS.\textsuperscript{52} Gel filtration of sera containing LATS or TSH showed that the two substances had different distribution coefficients in the gel and were recovered with different protein fractions. LATS was recovered in the 7S fraction, whereas TSH, whether endogenous or added in vitro, was found with the 4S fraction.\textsuperscript{59}

**Biological differences.** When TSH can be detected in serum its level can be decreased by the administration of L-thyroxine to the patient,\textsuperscript{1, 3} whereas LATS levels are not affected by such treatment.\textsuperscript{1, 66} Recently Foldes and co-workers\textsuperscript{38} claimed significant reduction of LATS levels in six patients taking D-thyroxine in relatively large doses but not while being treated with L-triiodothyronine.

When injected into experimental animals, the biological half-lives of the two stimulators are quite different. In the rat, LATS has a half-life of 7½ hours compared with that of 10 to 20 minutes for TSH.\textsuperscript{2, 56} A half-life of approximately two weeks for LATS in the newborn child has been inferred from data obtained in studies of congenital thyrotoxicosis.\textsuperscript{60}

**Evidence that LATS is not of pituitary origin.** An extra-pituitary origin for LATS is suggested both by direct and indirect evidence.

Extracts of the pituitary gland of patients who were known to have suffered from thyrotoxicosis during life\textsuperscript{65, 66} and of pituitary biopsies\textsuperscript{57} have yielded only conventional TSH activity. Furthermore, comparison of the LATS levels in jugular venous blood and in mixed systemic venous blood obtained simultaneously did not reveal an increased concentration of LATS in venous blood from the skull.\textsuperscript{68} LATS has been detected in the serum of hyperthyroid and euthyroid individuals suffering from hypopituitarism after hypophysectomy or pituitary stalk section.\textsuperscript{39, 50, 57} These results were published before the impor-
tance of nonspecific responses in the assay was realized, so that some of the lower responses are of doubtful significance, but in at least four of the cases the LATS responses were probably significant.

Assay of serum from some patients who became hypothyroid following I\(^{131}\) therapy for thyrotoxicosis showed an increased level of TSH, suggesting that pituitary function can react normally to thyroid deficiency in such patients. Other patients in this group have high levels of LATS in their sera.\(^6\) By using antisera to human TSH, Adams and Kennedy\(^6\) have shown that even when high levels of LATS persist in these circumstances the simultaneous presence of TSH may be demonstrated.

Indirect evidence is provided by reports of hyperthyroidism developing in certain hypopituitary patients after hypophysectomy,\(^13,\ 21\) pituitary stalk section,\(^30\) postpartum pituitary necrosis,\(^24\) and Y\(^90\) implantation in the pituitary for acromegaly.\(^96\) Thyrotoxicosis has also been reported in patients with chromophobe adenoma of the pituitary.\(^78,\ 100\)

Chemical characterization of LATS

Association with immunoglobulin G (IgG).\(^6\) The finding that LATS was recovered with the serum proteins after ultrafiltration and precipitation with organic solvents\(^74\) prompted studies of its distribution among the serum protein fractions. Both starch-block electrophoresis and ion-exchange chromatography on DEAE cellulose indicated that the thyroid-stimulating activity in active sera was distributed throughout the electrophoretic subdivisions of the serum proteins, although higher levels were usually associated with gamma globulin.\(^66,\ 74\)

The observation of Adams and Kennedy\(^6\) that LATS could be recovered in high yield with gamma globulin after cold ethanol precipitation stimulated more detailed studies of the association of LATS with this fraction. Gel filtration of active sera on G-200\(^37\) confirmed that LATS has associated with the 7S fraction of the serum proteins.\(^25,\ 58\) The preparations of gamma globulin obtained by these methods as well as by ammonium sulfate\(^33,\ 70\) and sodium sulfate precipitation\(^*\) were all active but not homogeneous. The isolation of LATS with immunochemically pure IgG from active sera has been described in reports from several laboratories.\(^28,\ 31,\ 45,\ 70\) In all these studies initial concentration of IgG was performed either by salting out\(^28,\ 45,\ 70\) or by ethanol precipitation\(^11\) prior to ion-exchange chromatography on either DEAE cellulose\(^28,\ 31,\ 70\) or DEAE Sephadex.\(^45\) Such preparations showed a tenfold increase in LATS activity per unit weight of protein compared with that of the original serum proteins and sedimented in the ultra-centrifuge as a single peak at a corrected rate \((S_{20, \text{w}})\) of 6.5/6.6S. Immunelectrophoresis and gel diffusion against anti-whole human serum showed only the single precipitation arc characteristic of IgG, and injection of purified preparations into rabbits produced monospecific antisera indicating the immunochemical homogeneity of the IgG. Recently Miyai and Werner\(^72\) isolated LATS-IgG from an active serum by DEAE-cellulose chromatography and subfractionated the IgG on CM cellulose. They demonstrated a heterogeneous distribution of LATS activity in the IgG subfractions and achieved a thirty to thirty-seven fold concentration of LATS, compared with that of the original serum.

The close association of LATS and IgG has been confirmed by neutralization studies using specific antisera. The LATS activity in both the purified preparations of IgG and unfractionated serum was annulled on incubation with antisera to human IgG.\(^20,\ 45,\ 70\) Antisera to human TSH were without effect on the purified LATS preparations.

While studying the effects of antisera to various serum protein fractions on LATS

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\(^*\)The nomenclature used for the immunoglobulins and their subfractions is that recommended by the World Health Organization.\(^22\)

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\(^*\)Dorrington and Munro: Unpublished observations.
activity, Dorrington and Munro\textsuperscript{30} found that an antiserum to IgA had potent anti-LATS activity. This unexpected finding may have been due to the presence of a high titer of antibodies to the light chain in the antiserum. The L chains of IgA and IgG are antigenically similar, which may account for the cross-reaction. Samples of parotid saliva, known to be rich in IgA,\textsuperscript{94} were collected from patients with high levels of LATS; the samples were inactive, indicating that IgA was unlikely to be the active fraction.\textsuperscript{*}

\textbf{Attempts to dissociate LATS from IgG.}\ Those who were prepared to concede that serum containing LATS was truly capable of stimulating thyroid function, but were less willing to accept that this could be due to an extra-pituitary factor, frequently favored the view that LATS could be TSH bound to serum protein. There is now chemical evidence to add to the other evidence suggesting that this is extremely unlikely.

Using techniques designed to disrupt the various types of noncovalent bonding between or within protein molecules, attempts have been made to dissociate the thyroid-stimulating activity of LATS from active IgG. Exposure of LATS-IgG to high concentrations of sodium chloride, urea,\textsuperscript{25, 58} guanidine HCl, or formic or acetic acids\textsuperscript{46} failed to dissociate any smaller active protein or peptide from the IgG, as judged by gel filtration on Sephadex G-200, and did not alter the time course of response in the bioassay.

\textbf{Proteolysis of LATS-IgG.} McKenzie\textsuperscript{59} studied the effects of various proteolytic enzymes on LATS activity in whole serum. He found that the LATS activity was not affected by trypsin, subtilopeptidase, pancreatic proteases, or papain. Extensive digestion with papain yielded a fraction in which the thyroid-stimulating activity (still long-acting) was retarded on a column of Sephadex G-25 indicating a molecular size of less than 5,000. These results have not been confirmed by more recent studies on the effects of papain and pepsin on LATS-IgG.

Purified preparations of LATS-IgG have been hydrolyzed with papain in a cysteine-EDTA activated system, according to the method of Porter,\textsuperscript{83} to yield three fragments of approximately equal size (Fig. 2). Although Porter's original experiments were performed on rabbit antibody, available evidence indicates that human IgG is similarly fragmented by papain.\textsuperscript{23} As with human nonspecific IgG and antibody, treatment with papain reduced the sedimentation rate from 6.6S to 3.5S.\textsuperscript{28} This reduction in molecular size from 150,000 to 50,000\textsuperscript{74} was accompanied by a dramatic change in the time course of thyroid stimulation in the mice used for bioassay.\textsuperscript{26, 70} The prolonged response typical of LATS was converted after proteolysis to a short-acting effect resembling TSH. This change was accompanied by a diminution of the peak response compared with the intact IgG. Further chromatographic analysis of the papain digest on DEAE cellulose separated the Fab-fragments from the F\textsubscript{c}-fragments. The thyroid-stimulating activity was located entirely in the Fab-fragments; F\textsubscript{c} was devoid of activity. Meek and co-workers\textsuperscript{70} found that only piece I (using Porter's\textsuperscript{83} nomenclature for rabbit IgG) was active, which suggests that LATS was present only in the electrophoretically slow fraction of IgG, since piece II is derived only from electrophoretically fast IgG.\textsuperscript{79} Unfortunately, piece II was only tested at half the concentration of piece I, which may account for the observed difference.

The change in time course of response following limited proteolysis of LATS-IgG has been subject to a number of interpretations. Meek and colleagues\textsuperscript{70} suggested that LATS was a thyrotropin-like substance bound to antibody, while Dorrington, Carneiro, and Munro\textsuperscript{26} felt that the change probably reflected a shorter half-life of the fragments compared with that of IgG as a consequence of the reduction in molecular size. Studies in this lab-
Fig. 2. Diagrammatic representation of IgG and the effects of reduction and proteolysis on the molecule. H, heavy chain; L, light chain. Values of the corrected sedimentation rates \((S_w, w)\) for the fragments are shown in parentheses. \(R = \text{CH}_2\text{CONH}_2\).

Laboratory have shown that in an in vitro system for the study of ¹³¹I release from whole mouse thyroids,⁰¹ F\(_{ab}\)-fragments caused a response equal to an equivalent concentration of LATS-IgG. These in vitro results, when renal and other clearance rates did not operate, indicated that proteolysis did not reduce the thyroid-stimulating activity of LATS-IgG to the extent suggested by the diminished in vivo responses.

Evidence has been obtained with specific antisera indicating that the thyroid-stimulating activity of F\(_{ab}\)-fragments remained antigenically distinct from TSH.⁰², ⁴⁶ The short-acting activity of a LATS-IgG papain digest was annulled on incubation with antisera to IgG and F\(_{ab}\) (but not anti-F\(_c\)), while antihuman TSH was without effect. Conversely, human TSH responses were only annulled by a homologous antiserum.

Similar findings have been reported with pepsin.⁰², ²⁸, ⁴⁶ In the absence of reducing agent, proteolysis of IgG with pepsin yielded a single fragment with a sedimentation rate of approximately 5S (molecular size 100,000), equivalent to two F\(_{ab}\)-fragments joined by a single disulfide bridge.⁰⁵ The portion of the molecule equivalent to F\(_c\) was extensively hydrolyzed by pepsin. Peptic digests of LATS-IgG retained their ability to cause prolonged stimulation of the thyroid in the McKenzie bioassay. However, the addition of a reducing agent, such as cysteine, lowered the sedimentation rate to 3.2S and, as after papain digestion, the time course of the assay response then became short acting. Thus, the F\(_{ab}\) and F\(_{ab}\)-fragments, produced from LATS-IgG by pepsin and papain, respectively, behaved identically when assayed. These two fragments appear to be similar on chemical analysis.⁶⁹ The results with pepsin provide further evidence that molecular size plays an important role in determining the time course of thyroid stimulation.

Reduction of LATS-IgG. As shown in Fig. 2, the heavy and light chains of IgG are covalently linked by disulfide bridges which may be cleaved by reduction in neutral aqueous buffers. Gel filtration of reduced IgG under conditions which mini-
mized noncovalent interactions resulted in the separation of two types of polypeptide chains. The heavy (or A) chains accounted for approximately 75 per cent of the reduced protein, and the light (or B) chains, for the remaining 25 per cent. The arrangement of the polypeptide chains in IgG is shown schematically in Fig. 2; the evidence for this structure has been reviewed by Cohen and Porter.23

Reduction studies on LATS-IgG by Meek and co-workers70 and by Dorrington, Munro, and Carneiro31 have yielded somewhat conflicting results. The latter group31 found that treatment of active IgG with 2-mercapto-ethanol and alkylation of the free sulfhydryl groups with iodoacetamide did not significantly reduce LATS activity nor change the time course of response. Meek and co-workers70 implied that there was a change to a short-acting response following reduction. Certainly, in their hands, the separated heavy chains gave a short-acting response in the assay and showed a twenty-five fold increase in specific activity. Both groups found light chains were inactive.

Using a similar technique, Dorrington, Munro, and Carneiro31 found that the separated heavy chains had only low levels of biological activity of doubtful significance. They also showed that the reduced LATS-IgG was only inactivated after dialysis against acetic or propionic acid. The native protein was quite stable to such treatment indicating a stabilizing role for the disulfide bonds. A more extensive study of the effects of reduction on LATS-IgG has recently been performed in the authors' laboratory.* A modified reduction and separation technique has been developed to curtail the time of exposure to the acid conditions required to separate the polypeptide chains. Heavy chains have been isolated with short-acting thyroid-stimulating activity which became long-acting on recombination with light chains. The light chains themselves were inactive. Approximately 15 per cent of the LATS activity of the original IgG was recovered after combination of the isolated heavy and light chains.

**Serum surveys**

After the discovery of LATS, some years elapsed before there were publications recording the examination of serum thyroid-stimulating activity in large series of patients with thyroid disorders and of normal subjects.68, 77, 80, 82 Because low levels of LATS were difficult to distinguish on assay from the nonspecific response, some claimed that LATS could be detected in the serum of normal subjects.68 However, it is now clear that, with the exception of occasional patients with exophthalmic ophthalmoplegia,69, 68 LATS can only be detected in the serum of patients with thyrotoxicosis or who have suffered from thyrotoxicosis at some earlier time. The frequency with which LATS has been detected in the serum of patients with thyrotoxicosis has varied, but has usually been between 40 and 60 per cent.68, 77

It is reasonable to postulate that most of the minor discrepancies in such observations arise from slight modifications in the McKenzie bioassay in different laboratories and from the lack of an agreed international standard for LATS to control assay sensitivity (see below). Patients with exophthalmic ophthalmoplegia are of particular interest because it has been known for some years97 that, just as in untreated thyrotoxicosis, thyroid function may not be suppressed in this group after the ingestion of large doses of exogenous thyroid hormone. So far, there has been no report of direct observations made to test the hypothesis that failure of suppression of thyroid function by exogenous thyroid hormone in thyrotoxicosis or exophthalmic ophthalmoplegia is associated with LATS. The patient described by Major and Munro68 with severe exophthalmic ophthalmoplegia and high serum LATS, although euthyroid, had a rapid turnover of I131 in a tracer study, in spite of normal chemical protein bound iodine.

*To be published.
Only McKenzie has examined serum from a substantial number of patients with 
solitary toxic adenoma of the thyroid causing thyrotoxicosis, and his results sug-
ggest that LATS cannot be detected in un-
concentrated serum under these circum-
stances. It is, however, possible to detect 
LATS in the serum of patients with thyro-
toxicosis with either nodular or generalized 
enlargement of the thyroid.

LATS and the eye signs of thyrotoxicosis

There has been considerable speculation 
about a possible etiological role for LATS 
in relationship to the dramatic eye signs 
which occur in some patients with thyro-
toxicosis. The cause of these eye signs has 
remained very obscure in spite of much 
work on the exophthalmic producing sub-
stance (EPS) which may be detected in 
animal pituitary extracts. Dobyns, 
Rudd, and Wright and McGill have 
claimed to detect EPS in the serum of 
patients with severe exophthalmic ophthal-
moplegia. The observation quoted above 
that LATS may also be found in such pa-
tients' sera has led to much interest in the 
relationship between LATS and EPS.

The balance of evidence suggests 
strongly that they are not identical. This 
opinion is based both on serum fractiona-
tion studies which separated the two activi-
ties and on the failure of both Major and 
Munro and McKenzie to find any con-
sistent relationship between the severity 
of the associated eye involvement in thyro-
toxicosis and serum LATS. Difficulties in 
studying this problem arise from the in-
constant relationship between the progress 
of the eye signs and excessive thyroid 
activity in thyrotoxicosis. It is well recog-
nized that deterioration in eye signs is not 
necessarily associated with increasing thy-
roid activity, and this also suggests that 
the etiological factors responsible for these 
two aspects of Graves' disease differ.

Nevertheless, Kriss, Sharp, and Utiger have 
recently reported a correlation be-
tween the development of ophthalmopathy 
in Graves' disease and the detection of 
serum LATS. It may well be that more 
serial serum assays in individual patients 
with fluctuating eye signs will clarify this 
point as earlier observations have usually 
been limited to assaying LATS in single 
serum samples. However, Major failed to 
detect serum LATS during a period of 
rapid deterioration in the eye signs of a 
patient with Graves' disease.

The association of LATS with localized 
myxedema

Pimstone, Hoffenberg, and Black detected high levels of LATS in serum from 
patients with localized pretibial myxedema and extracted low levels of long-acting 
thyroid-stimulating activity from biopsies of the abnormal skin.

Kriss, Pleshakov, and Chien have em-
phasized that very high LATS levels are 
frequently found in the serum of patients 
who have developed localized myxedema 
as a complication of thyrotoxicosis. This 
has led them to adopt the opinion that 
LATS is a secondary phenomenon occur-
ing in these rare cases and not of general 
importance in the pathogenesis of thyro-
toxicosis. It is difficult to accept this limited 
role for LATS in patients with thyro-
toxicosis in view of the evidence that it is 
a true stimulator of thyroid function in 
animals and man, and that there is a cor-
relation between the level of LATS in 
serum and the thyroid overactivity in pa-
tients with thyrotoxicosis. Furthermore, 
in all large series there are patients with 
high serum LATS levels who never de-
veloped localized myxedema, and not all 
patients with this complication necessarily 
have high levels of LATS. Nevertheless, 
the observation that localized myxedema 
is a good "marker" for a high serum LATS 
level has proved to be of great use to all 
workers in this field.

LATS and excessive thyroid function in 
thyrotoxicosis

All the reported serum assays from 
groups of patients with thyrotoxicosis have 
included a substantial proportion of sam-

amples which could not be shown by the McKenzie assay to contain LATS. This has inevitably led to considerable doubt about the role of LATS in the pathogenesis of thyrotoxicosis. There are now two lines of evidence which suggest that these doubts are not justified. First, concentration procedures for LATS, when applied to sera which cannot be shown to contain LATS when originally assayed, have established that the majority of inactive sera do in fact contain LATS, so that the failure with unconcentrated sera probably reflects only the relative insensitivity of the assay method.

Other supporting evidence for the view that LATS causes the increased thyroid activity of thyrotoxicosis comes from the significant correlation between the radioiodine turnover rate and the LATS level in serum. The correlation with iodine turnover rate has now been confirmed in a larger series of patients; furthermore, when gland size was estimated by a group of experienced observers there was also a significant correlation between LATS level in the serum and the mean estimate of thyroid mass. The closest correlation, however, was found when serum LATS was related to the rate of iodine turnover per unit weight of thyroid. A correlation between serum LATS levels and the histological hypertrophy of the thyroid acinar cells has also been reported. These findings can leave little doubt that LATS is directly responsible for the increased thyroid activity of thyrotoxicosis.

**LATS standard**

A major difficulty in comparing LATS measurements in different centers has been the absence of a suitable standard. Neither is there a potent tissue source of LATS which could be extracted for this purpose, nor can LATS be produced in experimental animals. Thus, the only possible way of establishing a standard has been to obtain exceptionally potent sera from patients. Such a standard was established by Dor-}

**The interaction of LATS with thyroid tissue in vitro**

The accumulated evidence that LATS activity is an inherent property of specific IgG molecules has stimulated investigations into the possibility that LATS may be an antibody. This hypothesis has been tested by observations on the interaction between LATS and thyroid tissue in vitro. Kriss, Fleschakov, and Chien incubated serum with homogenates of thyroid or other tissues, including liver and kidney, from the dog. Some loss of LATS was observed after incubation with all the homogenates tested, except spleen, but the loss was tenfold higher with thyroid tissue. These workers also studied the absorption of LATS by human thyroid slices and demonstrated a progressive loss of LATS activity until, by 24 hours, 76 per cent of the original activity had disappeared.

In contrast, Pinchera, Pinchera, and Stanbury failed to demonstrate any loss of LATS when serum was incubated with human thyroid slices or cell-membrane preparations. Similar negative results were also obtained with human and bovine TSH. These negative findings may have been related to a high level of LATS in the patients from whom the thyroid tissue was obtained. Thus, the number of free “binding sites” for LATS would be small.

More recently, El Kabir and co-workers have studied the organ specificity of LATS...
absorption in more detail. They confirm that homogenates of human thyroid obtained at operation and autopsy absorbed LATS from serum, although fresh nontoxic nodular goiters proved more effective in their hands. Among the other human tissues tested, 5 mg. per milliliter of post-mortem kidney halved the LATS activity, but no further decrease could be obtained even though the amount of kidney homogenate was increased two hundred fold. Similar, although less dramatic, results were obtained with fresh skeletal muscle and gastric mucosa. These workers were unable to account adequately for this partial absorption by tissues other than thyroid or for the failure of this activity to increase with increased tissue concentration. The possibility of the thyroid absorption sites sharing some determinants with sites in other tissues cannot be excluded.

Dorrington, Carneiro, and Munro could not demonstrate significant absorption of LATS-IgG by homogenates of human myometrium under conditions in which thyroid preparations showed maximal absorbing activity.

At least two groups have investigated the subcellular localization of the LATS binding sites using standard differential ultracentrifugation techniques. Homogenates of human thyroid tissue obtained at operation or autopsy were fractionated to give nuclei (800 g fraction), mitochondria (10,000 g fraction), and microsomes (100,000 g fraction). Although all the fractions absorbed LATS either from whole serum or from purified IgG, the microsomal fraction showed the highest activity. Since the subcellular fractions obtained by differential ultracentrifugation are not homogeneous, the partial absorption by the nuclear and mitochondrial fractions might have been due to microsomal contamination, as repeated washing of these two fractions reduced their absorbing capacity. Absorption of LATS was specific for thyroid microsomes; preparations from a variety of other tissues were uniformly inactive. This implies that the non–organ-specific absorption described by El Kabir and co-workers must be due to sites located in another subcellular region.

Beall and Solomon have studied some of the physical and chemical characteristics of thyroid microsomes in relation to their LATS-absorbing ability which was destroyed by heating above 55° C. or by treatment with deoxycholate, urea, acid, or alkali. The absorption of LATS was not affected by a number of enzyme inhibitors. These workers have not been able to extract the LATS-absorbing fraction from the microsomes by freezing and thawing or by ultrasonic vibration. Incubation, or freezing and thawing, of microsomes in normal human serum or of bovine serum albumin released a small amount of inhibitory activity into the supernatant after centrifugation at 100,000 g.

Dorrington, Carneiro, and Munro found that when the microsomal fraction was treated so as to separate the ribonuclear protein (RNP) particles from the endoplasmic reticulum, the LATS absorption activity remained with the membrane fraction and could be sedimented at 100,000 g.

LATS has been successfully eluted from microsomes under acid conditions, indicating that absorption did not result in inactivation of the molecules in vitro. This finding should allow separation of the specific LATS-IgG from other IgG molecules thus allowing maximum purification. Suspensions of microsomes with absorbed LATS did not cause any thyroid stimulation when injected into assay animals, indicating that the active portion of the IgG was blocked. Short-acting Fab-fragments from LATS-IgG were also absorbed by microsomal preparations, indicating that the absorption site on the molecule is located at, or near, the thyroid stimulating site.*

Parallel studies on the absorption of TSH by microsomes have yielded conflicting results. Dorrington, Carneiro, and Munro could not demonstrate absorption of human TSH either by thyroid homoge-

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*Unpublished observations.
nates or subcellular particles. Beall and Solomon, inhibited bovine TSH by incubation with thyroid microsomes, but they also showed that the assay response to TSH (but not LATS) was inhibited by low concentrations of thyroxine. Therefore, the inhibition of TSH by microsomes may have been due to elution of iodothyronines or iodoprotein from the microsomes during incubation.

The possibility of localizing the binding sites for LATS-IgG within the thyroid gland with the use of fluorescent labeling techniques has received some attention, although the results have been disappointing. Kriss and co-workers, using the fluorescence technique of Greenspan and Haragadine, reported that LATS-IgG was localized in the thyroid cell nucleus. In unpublished work Dorrington, Munro, and Taylor, and Roitt, using a sandwich technique with fluorescein labeled rabbit antihuman IgG, were unable to demonstrate any localization of LATS-IgG in frozen thyroid slices. Attempts to localize LATS in the thyroid with use of ferratin labeling have not, thus far, been reported.

The large molecular size of LATS-IgG (about 160,000) would make it doubtful that the thyroid-stimulating activity would have free access, in vivo, to sites on the endoplasmic reticulum. As there is considerable doubt whether IgG with specific antibody activity is capable of penetrating viable cells, there is no a priori reason to endow LATS with this property. As the endoplasmic reticulum appears to be continuous with the outer cell membrane, the LATS-absorbing properties of the microsomal fraction may be attributed to fragments of cell membrane present in the fraction. It would be of great interest to prepare cell membranes by the osmotic rupture of thyroid cells and study their LATS-absorbing ability.

Is LATS an antibody?

Current hypotheses about the nature of LATS and its interaction with the thyroid must be assessed in relationship to a possible autoimmune etiology for Graves' disease. The evidence for the antibody nature of LATS is reviewed below.

**Chemical comparison of LATS and known antibodies.** The close association of LATS with IgG has been reviewed in an earlier section. All available evidence indicates that the thyroid-stimulating activity is an inherent property of specific IgG molecules, although it remains difficult to provide absolute proof that the activity may not be due to a small peptide tightly bound to IgG.

Much is now known concerning the localization of antigen-binding sites in antibody molecules. Chemical and enzymatic degradation of IgG with specific antibody activity has clearly indicated that the binding sites are located within the F\(\_\text{ab}^\prime\)-fragments. Similar localization of thyroid-stimulating activity has now been established for LATS-IgG. The relative contributions of the heavy and light chains, isolated following reduction of IgG, to antibody activity remains a matter of some controversy. Some workers have provided evidence that antigen-binding activity is a property solely of the heavy peptide chain, while others contend that full expression of antibody activity requires the presence of light chains. As far as LATS-IgG was concerned, some thyroid-stimulating activity was recovered in isolated heavy chains but light chains were inactive. It is difficult to decide whether the change in the time course described, following the addition of light chains to active heavy chains, represented an enhancement in activity or merely reflected the reassociation of the peptide chains to form 7S molecules. Despite this reservation, the similarities between antigen binding in antibodies and thyroid-stimulating activity in LATS-IgG are very striking. Combining available information, the likely site for the active portion of LATS-IgG is the F\(\_\text{d}\)-fragment (Fig. 2) with possibly some auxiliary contribution from the light chains. Recent efforts in this laboratory have been directed toward the demonstration of activity within the F\(\_\text{d}\)-fragment of LATS-IgG.
The synthesis of LATS by cells of the lymphoid system. Until very recently, except for the claim that LATS could be detected in extracts of localized myxedema, the only known source of LATS was serum. The realization that LATS may be an immunoglobulin prompted the study of LATS production by lymphoid tissue, which is known to be the site of IgG synthesis.

McKenzie and Gordon cultured white blood cells from the blood of patients with LATS. In the presence of phytohemagglutinin, which is known to stimulate IgG synthesis by lymphocytes, low levels of LATS activity were detected in the supporting medium. The culture fluid was changed daily for up to seven days, and 7S protein was concentrated by gel filtration on Sephadex G-200 before assay. Incorporation of C14 amino acids into gamma globulin isolated from the cultures by electrophoresis confirmed that synthesis of IgG proceeded in this type of system.

In view of the low levels of LATS response obtained, more work needs to be done along the lines initiated by McKenzie and Gordon to achieve completely convincing responses on assay. All white cells do not produce antibody and in McKenzie and Gordon's cultures only a small proportion could be expected to synthesize LATS-IgG. It is, therefore, doubtful if really significant LATS responses could have been expected, particularly as the size of a series of cultures must be limited by the volume of blood which can be withdrawn from a donor. Possibly lymph node tissue, obtained after a thyroidectomy, from patients with high levels of LATS would prove a more fruitful source of LATS-producing cells.

The suppression of LATS production by corticosteroids. Kriss, Pleshakov, and Chien found that oral corticosteroid therapy combined with local application of corticosteroid cream to leg lesions, in two thyrotoxic patients with pretibial myxedema and exophthalmos, resulted in an initial sharp rise in serum LATS followed by a progressive fall to a quarter of the pretreatment level. Improvement in the pretibial myxedema accompanied this partial disappearance of LATS. The proptosis increased in severity while the LATS level was falling. Interruption of steroid treatment resulted in progressive deterioration of the localized myxedema, accompanied by a rise in LATS titer to a level above the pretreatment value. A recent preliminary communication from Kriss, Sharp, and Utiger suggests that LATS levels rise as eye signs deteriorate. A decrease in LATS level during treatment with prednisolone has also been observed in three patients by Green, Snyder, and Solomon, accompanied by a remission of eye signs in two patients. Werner and Platman have claimed that remission of thyrotoxicosis may be induced by steroid therapy, but these authors did not measure LATS in the group of patients studied. These results can be quoted as a demonstration of the well-known immunosuppressive action of corticosteroids. The early rise in LATS on commencement of treatment described by Kriss, Pleshakov, and Chien may have been due to a sudden release of IgG following widespread breakdown of lymphocytes. The cessation of steroid treatment would have resulted in a gradual recovery of the lymphoid cells and recommencement of antibody synthesis, thus accounting for the observed rise in LATS titer.

Occurrence of autoantibodies in thyrotoxicosis. Patients with thyrotoxicosis exhibit disturbances of immune tolerance. Autoantibodies to components of thyroid tissue occur in thyrotoxicosis as well as other thyroid diseases; the relatives of patients have also been shown to have an unusually high incidence of such antibodies.

The presence of autoantibodies to tissues other than thyroid has also been demonstrated in the serum of thyrotoxic patients more frequently than in control subjects. The incidence of antibodies to gastric parietal cell microsomes and to intrinsic factor...
in thyrotoxicosis has been studied\(^4\),\(^9\); overt and latent pernicious anemias have been found more frequently in thyrotoxic patients than in matched control subjects.

A higher incidence of diseases associated with disturbances of immune tolerance, such as pernicious anemia and rheumatoid arthritis, occur in the relatives of thyrotoxic patients than would be expected in those from random association.\(^1\)

Local lymphocytic infiltration of the thyroid in thyrotoxicosis has been described,\(^4\) and more recently Irvine and co-workers\(^4\) have reported thymic enlargement in patients with high titers of thyroid antibodies. A clearer understanding of the function of the thymus in the adult is required before the significance of the latter finding can be assessed.

It is perhaps of interest that Halsted\(^3\) more than 50 years ago claimed that remission of Graves' disease followed surgical removal of the thymus.

The occurrence of disorders of immunological tolerance, to which there may be genetic predisposition, in thyrotoxicosis does not provide direct evidence that LATS is an antibody. Nevertheless, it may be concluded that the probability of auto-antibody formation in thyrotoxic individuals is higher than in other groups. It would be of interest to examine the serum of relatives of patients with thyrotoxicosis for the presence of LATS.

The passive transfer of LATS activity. One of Milgrom and Witebsky's\(^7\) requirements for establishing an autoimmune etiology for any disease is that it can be passively transferred with serum or immunologically competent cells. Some features of congenital thyrotoxicosis fit this requirement. Children of mothers with high levels of LATS may be born with a transient form of hyperthyroidism which remits spontaneously during the first three months of life. LATS has been found in the serum of such children, and it disappears with a half-life approximately the same as the half-life of IgG.\(^6\) These observations are completely compatible with the known placental transfer of IgG (but not other immunoglobulins), and the gradual postnatal disappearance of IgG from the serum of the child coincides with the development of immunological competence.

The development of hyperthyroidism when serum from thyrotoxic patients was infused into normal subjects\(^1\) provides further evidence that effects of LATS can be passively transferred.

Summary of evidence

Taken as a whole, the evidence cited above strongly supports the hypothesis that LATS is an antibody. Considered in isolation, the chemical evidence is the strongest although it is not clear whether IgG exists without antibody activity. Animals reared under sterile conditions have low but significant levels of immunoglobulin with an unknown function.

If LATS is an antibody, then a complementary antigen must exist, and the efforts directed at characterizing this, as yet, hypothetical entity have been described.

The demonstration of LATS-IgG absorption by the microsomal fraction of thyroid cells poses the question: Does this phenomenon mimic the hypothetical antibody-antigen reaction which results in stimulation of the intact thyroid cell? Current efforts in several laboratories are directed toward raising antibodies, in rabbits, to human thyroid cell microsomal fractions. The resultant antisera, at least in the authors' laboratory, have not, so far, been shown to have unequivocal LATS activity. In view of the ability of LATS to stimulate thyroid function in man and several experimental animals, including the rabbit,\(^4\) these experiments may fail because of immune tolerance toward the antigen, which will probably be very similar in all species. Nevertheless, antibody formation in experimental animals following injection of homologous thyroid extracts is well known.\(^9\)

If the experiments in progress are suc-
ccessful, a considerable advance in establishment of the antibody nature of LATS will have been achieved.

**Speculations on the nature of the antigen**

The evidence that LATS is an antibody has prompted speculation about the nature of the antigen with which it reacts in the thyroid.\(^6\) \(^{25}\) \(^{45}\) \(^{62}\) On the basis of the evidence that LATS and TSH have similar effects on the thyroid, it has been suggested that in thyrotoxicosis an unidentified TSH receptor becomes antigenic following a breakdown in immune tolerance. It is further postulated that the resultant antibody, LATS, has combining sites similar to the active groupings in TSH and is thus capable of causing unregulated thyroid stimulation, free from the control of the pituitary-thyroid feedback mechanism.\(^6\) \(^{25}\)

Alternatively, it has been suggested\(^45\) \(^{62}\) that the antigen is a genetic repressor required for the regulation of normal thyroid function, when inactivated uncontrolled thyroid activity results. The finding\(^45\) that pretreatment of the assay animal with either actinomycin D or puromycin suppressed responses to LATS and TSH suggested that both stimulators influence either RNA synthesis or RNA-directed protein synthesis. It must be remembered, however, that both these antibiotics are very toxic agents and the specificity of their action in whole animal may not be assumed in the absence of corroborative evidence.

The genetic repressor hypothesis may be criticized as yet another example of the widespread and often unjustified extension of the theories of Monod.\(^73\) The possibility that LATS interacts with an allosteric enzyme system or is an antibody to an allosteric inhibitor provides an alternative hypothesis equally in accord with modern concepts of metabolic control.

As commented upon in the above, it is doubtful whether LATS could act efficiently, except at the cell surface, in view of its molecular size. In these circumstances the antibody to a TSH-receptor theory is the most attractive, but it does not exclude secondary effects at the level of the gene. More knowledge will probably be needed concerning the details of the response of the thyroid gland to TSH stimulation before any detailed mechanism for LATS action can be formulated.

It is clear that in many ways LATS is still an enigma, despite the concentrated efforts of many research groups. As LATS may be the first good example of an antibody capable of stimulating specific cellular function, its nature will become of increasing interest to others not directly concerned with its role in the etiology of Graves' disease.

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