Section of Clinical Immunology and Allergy

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The Long-acting Thyroid Stimulator (LATS) [*Abridged*]

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Observations on the Nature and Significance of the Long-acting Thyroid Stimulator

Adams & Purves (1956) discovered the long-acting thyroid stimulator (LATS) which is now widely believed to be of importance in the pathogenesis of Graves' disease (McKenzie 1968). In the commonly used McKenzie (1958) bioassay, LATS causes a sustained release of radioiodine from the thyroid glands of mice which reaches its maximum 10–12 hours after the intravenous injection of serum from patients with Graves' disease. This contrasts with the relatively short-lived effect of pituitary thyroid-stimulating hormone (TSH) which reaches a maximum at 2 hours, thus enabling TSH to be distinguished from LATS.

Interest in LATS quickened when it was established that it could not be separated from pure γG and, after enzymatic hydrolysis, the thyroid-stimulating activity was located in Fab or Fab′ fragments (Dorrington *et al.* 1965). Separation of heavy and light chains from LATS-γG showed the active groups to be in the heavy chain and confirmed that the duration of action of active fragments was related to molecular size. This was most convincingly demonstrated by experiments in which heavy chain, with short-acting thyroid stimulatory activity, was recombined with inactive light chain and the time course of assay response reverted to the characteristic long action of LATS (Munro *et al.* 1967).

Although LATS may usually be completely recovered from serum with γG prepared by the standard method of chromatography of pseudoglobulins on DEAE-cellulose, there are occasional patients in whom all the thyroid-stimulating activity is lost when this method is used. Detailed study of selected potent sera has shown that these losses result from the association of LATS, to a variable extent, with γG which is relatively insoluble at low ionic strengths and tends to precipitate when γG preparations are dialysed (Munro *et al.* 1967). In keeping with this evidence for a heterogeneous distribution of LATS among γG molecules, Smith (to be published) has shown by chromatography of whole serum on DEAE-sephadex, using gradient elution with low molarity phosphate buffer, that LATS activity was asymmetrically distributed amongst the different electrophoretic subfractions of γG. Thus, from several different approaches, chemical evidence has revealed very close similarities between LATS-γG and known antibodies. Several thyroid auto-antibodies have already been characterized in Graves' disease so that it is necessary to consider whether LATS may also belong to this category of substances.

In spite of there being no clear precedent for the stimulation of an organ by an auto-antibody, there can be little doubt that LATS is the ultimate cause of the increased thyroid activity in Graves' disease (Carneiro *et al.* 1966) and several mechanisms have been proposed (*see* McKenzie 1968).

Much recent work has been concerned with the search for an intrathyroidal antigen by studying the selective absorption of LATS by homogenates and subcellular fractions of human thyroid tissue (Benhamou-Glynn *et al.* 1967). This topic, and the

results of immunization of rabbits with thyroid extracts which inhibit LATS is discussed by Dr Beall & Dr Solomon (this page) and by Dr Benhamou-Glynn and her colleagues (p 1303).

An alternative method for studying the interaction of LATS and the thyroid became available when Brown & Munro (1967) described a new system for the maintenance of mouse thyroids *in vitro*. In this bioassay TSH and LATS cannot readily be distinguished as their time courses of action are identical and the dose-response lines do not differ significantly from parallelism. The proportions of labelled thyroxine and triiodothyronine in the system increase in a similar manner with increasing concentrations of TSH and LATS (Ensor, to be published).

Ensor & Munro (1967) have published a preliminary report on the *in vitro* actions of cyclic 3',5' adenosine monophosphate (cyclic AMP) on the thyroid. This study has been extended by observing that theophylline (a known inhibitor of phosphodiesterase) potentiates the effects of TSH, LATS and cyclic AMP. This suggests that the three materials share a common pathway for thyroid stimulation.

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Thyroid Stimulating Factor in the Serum of Immunized Rabbits

Several investigators have reported that rabbits immunized with thyroidal extracts develop thyroid-stimulating activity in their serum (Pinchera *et al.* 1966, McKenzie 1967, Beall & Solomon 1968), supporting the idea that LATS may be an antibody. This paper will present a summary of the studies we have made of this phenomenon.

The McKenzie bioassay in mice was used to assay thyroid-stimulating activity. Immunization of rabbits was carried out with a human thyroid microsomal fraction in Freund's adjuvant. Details of procedure can be found in the references cited.

Fifty-one rabbits were immunized with human thyroid and 32 developed thyroid-stimulating activity in their serum. This activity was usually greatest one or two weeks after the termination of the 10-week immunization period and declined rapidly in the following weeks. A prompt increase in the amounts of thyroid stimulator found in the serum followed repeated immunization.

The rabbits produced many antibodies in response to the immunization. Precipitins to human serum proteins and thyroglobulin, complement-fixing antibodies to microsomes, hæmagglutinins to thyroglobulin and antibodies to thyroid colloid and cytoplasm were regularly produced in high titre. There was, however, no correlation between titres of any of these antibodies and the thyroid stimulator.

Thyroid function of the immunized rabbits was apparently normal since they gained weight normally, appeared to be well and the thyroidal ^{131}I uptake was not changed. Other thyroid function tests were confused by the appearance of an antibody which bound ^{131}I-thyroxine in the γ-globulin region of a paper electrophoretic strip. This is apparently the same phenomenon as that described by Premachandra *et al.* (1963) in guinea-pigs. As a result of this binding the immunized rabbits had elevated protein-bound iodine and thyroxine values and the resin uptake of ^{131}I-triiodothyronine was reduced.

The thyroids of 9 of the animals were examined histologically: there was no recognizable evidence of thyroiditis.

We also studied the functional, chemical and immunological nature of this thyroid stimulator in immunized rabbit serum (IRS). Comparisons were made with human LATS and rabbit TSH (myxœdematous rabbit serum). The IRS produces thyroid stimulation in mice intermediate in action between TSH, with its peak activity at 2 hours, and LATS, with peak activity at 8 hours or later. The activity of the IRS was usually greater at 2 hours than at 8 but the 2-hour to 8-hour ratio was significantly different from that of simultaneously assayed rabbit or bovine TSH. This finding suggested that the stimulator in IRS may be a mixture of TSH and a longer-acting stimulator. This hypothesis was supported by studies with anti-TSH serum since such a serum neutralized the 2-hour activity of IRS but left the 8-hour activity unaffected. DEAE-cellulose