LONG-ACTING THYROID STIMULATOR (LATS) IN TOXIC NODULAR GOITRE, TOXIC ADENOMA AND GRAVES' DISEASE

By
B.-A. Lamberg, A. Gordin, M. Viherkoski and G. Kvist

ABSTRACT

The long-acting thyroid stimulator (LATS) was determined by means of the McKenzie assay in 68 patients with hyperthyroidism. The patients were classified into the following groups:

Group 1. Graves' disease (diffuse goitre with hyperthyroidism with or without exophthalmos). Of the 35 patients tested 25 (71 %) were LATS-positive.

Group 2. Graves' disease with nodular goitre (nodular goitre with hyperthyroidism and with exophthalmos). The present series includes only 4 such patients, although this combination is by no means uncommon in Finland. Two of the patients were LATS-positive. It has been suggested that these patients represent Graves' disease superimposed upon endemic nodular goitre.

Group 3. Toxic nodular goitre. The present series comprises 23 patients with toxic multinodular goitre, of whom 10 (44 %) were LATS-positive. In view of the findings on thyroid palpation, on thyroid scintigraphy, the presence or absence of LATS in the blood and some other criteria, these patients can be divided into two categories, (a) one with Graves' disease superimposed upon nodular goitre of endemic origin (see group 2) and (b) the other with classical multinodular goitre. Analysis of the scintigrams showed that in some patients (with either exophthalmos or LATS in the blood and nodular goitre = Graves' disease + nodular goitre) it was not the nodules that were activated but the paranodular tissue, a finding which gave a scintigram typical of patients with classical Graves' disease. In some LATS-positive cases, however, some nodules were also activated to the same extent. The difference between these scintigrams and those typical of classical multinodular goitre is particularly stressed
since in Finland «toxic nodular goitre» is the prevailing type of hyperthyroidism.

*Group 4.* Single toxic adenoma. Two patients out of 6 were LATS-positive. This is in contrast to the findings of other authors according to which LATS has never been found in patients with toxic adenoma. A hypothesis is put forward that in these patients subclinical Graves' disease (LATS in the blood) coincided with a primarily autonomous, hyperactive but not necessarily toxic single thyroid adenoma, which was more susceptible to the stimulating activity of LATS than the surrounding tissue.

In previous studies it was shown that in Finland only 10–20% of the patients with hyperthyroidism have classical Graves' disease with or without infiltrative exophthalmos but with diffuse goitre, whereas the remaining 80–90% have nodular goitre (Wahlberg 1938; Järvinen & Leikola 1955; Lamberg et al. 1958).

Most of the patients with hyperthyroidism and nodular goitre were believed to have classical toxic nodular goitre, whereas a smaller group of patients, those with exophthalmos, probably had Graves' disease superimposed upon the endemic nodular goitre commonly found in Finland.

In a large study on thyroid histology Wahlberg (1933) stated that it was difficult even on histological grounds, to draw a clear line between Graves' disease proper and the toxic nodular goitre found in Finland. Since thyroid scintigraphy has come into wider use a clearer view has emerged. It appears that about 35% of the patients with hyperthyroidism have Graves' disease proper, 30% a single toxic adenoma and 35% toxic multinodular goitre (Kvist, unpublished data). The group denoted Graves' disease includes patients with classical Graves' disease and patients with infiltrative exophthalmos and a multinodular goitre.

The long-acting thyroid stimulator, LATS, has been regarded as a sign of the presence of Graves' disease, being positive in some of 50–70% of cases.

Only a very small number of patients with toxic multinodular goitre have been tested and some have been found LATS-positive though it is impossible to evaluate the actual incidence.

LATS has also been looked for in some 30 to 50 cases with a single toxic adenoma, and found to be absent in all of them.

For the above reasons it seemed to be of great importance to study the incidence of LATS in hyperthyroid patients in Finland, where hyperthyroidism mainly occurs in connection with nodular goitre.

**MATERIAL AND METHODS**

*Patients*

The study comprises 68 hyperthyroid patients from the Third Department of Medi-
cine and the Endocrine Out-Patients' Department, University of Helsinki, and ten healthy subjects. The patients were grouped, as shown in Table 1, according to the diagnosis: 1) Graves' disease: Hyperthyroidism and diffuse goitre with or without infiltrative exophthalmos. 2) Graves' disease with nodular goitre: Hyperthyroidism, nodular goitre and infiltrative exophthalmos. 3) Toxic nodular goitre: Hyperthyroidism, multinodular goitre with no signs of infiltrative exophthalmos. 4) Toxic adenoma: Hyperthyroidism and a single, scintigraphically hyperactive nodule, no uptake of radioactivity by the paranodular thyroid tissue, and no signs of infiltrative exophthalmos.

The diagnosis of hyperthyroidism was based on the clinical picture and usual laboratory data (Lamberg 1967). The presence or absence of nodules in the thyroid was evaluated on the combined selective use of careful thyroid palpation, thyroid scintigraphy and in addition, in the patients subjected to thyroidectomy, of a macroscopic and microscopic examination of the gland (Department of Pathology, University of Helsinki).

The mean age of the patients in each group is shown in Table 1.

Methods

The laboratory tests used for the diagnosis of thyroid function included determination of serum protein-bound iodine (PBI), free proportionate and absolute thyroxine in the serum, \( T_3 \)-Sephadex uptake, radioactive iodine tests, serum cholesterol, erythrocyte glucose-6-phosphate dehydrogenase activity, plasma and urinary hydroxyproline, etc. (Lamberg 1967).

Thyroid scintigraphy was usually carried out (Kvist, unpublished data) using the automatic scanning device of Nukab (Nukleonik Instrument AB, Gothenburg, Sweden), 24 h after administration of 50 \( \mu \)Ci Na\(^{131}\)I. All detected counts as well as the coordinates were recorded on a magnetic tape. After the study the tape was replayed and the activity visualized on an oscilloscope screen from which polaroid pictures were taken. By taking several pictures at varying degrees of activity subtraction and contrast amplification, it was possible to obtain adequate information about the activity distribution.

In a small minority of cases the scintigrams were obtained with the Picker Magna-scanner III (Picker X-ray Corp., Cleveland, Ohio, U. S. A.).

LATS was determined by a modification of the McKenzie assay (McKenzie 1958; McKenzie & Williamson 1966). Female mice weighing 16–20 g were used. The animals

<table>
<thead>
<tr>
<th>Table 1. Results of determination of LATS.</th>
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<tr>
<td>Group of patients</td>
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<tr>
<td>1) Graves' disease</td>
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<tr>
<td>2) Graves' disease with nodular goitre</td>
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<tr>
<td>3) Toxic nodular goitre</td>
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<td>4) Toxic adenoma</td>
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<td>5) Healthy subjects</td>
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were kept for about two weeks on an iodine-deficient diet before use. Thereafter they were given intraperitoneally a dose of 10 μCi 131I, and 10 μg of L-thyroxine subcutaneously at the same time. Forty-eight hours later, another subcutaneous injection of 10 μg of L-thyroxine was administered and after a further 48 h 0.5 ml of the serum to be tested was injected intraperitoneally (Kriss et al. 1964; Rerup & Melander 1965). For each serum 4–6 animals were used and each series included similar groups of animals which received 0.1–1.0 mU of a standard TSH preparation (NIH-TSH 4 USP units/1 mg). Blood was withdrawn with a Carlsberg pipette from the retrobulbar venous plexus at 0, 3 and 24 h after the injection of the serum sample, or of TSH. The radioactivity in 100 μl of blood was measured in a well-type scintillation counter.

The serum samples were stored in a deep-freeze at −20°C until used.

Control studies

For assessing the validity of the test, control series were studied at intervals after the injection of saline and albumin solutions or serum from healthy subjects. The radioactivity in the serum of the mice 24 h after the injection of these test solutions, showed a decrease as compared with the zero-hour value; the response varied from 60–100 %, with a mean of 73 ± 19.7 (mean ± sn) when the zero hour value was regarded as 100 %.

Evaluation of the response. According to the literature an increase of the counting rate to 200 % (i.e. doubling) has usually been regarded as a significantly positive response to LATS 9–24 h after the administration of the sample containing LATS (McKenzie & Williamson 1966). In some studies a variation of up to 150 % has been reported to occur in the control series. In the present study a response of 200 % or more was regarded as definitely positive. Since, however, the response in the control series remained below 100 % it seemed justifiable to regard even a response of over 150 % as positive. No attempts at statistical evaluation without or after log transformation were made in the present study, as has been suggested in the literature (Sakiz & Guillemin 1965; McKenzie 1967). A higher value at 24 h than at 3 h was regarded as indicative of a LATS reaction and the reverse of TSH.

RESULTS AND DISCUSSION

1) Graves' disease. Of the 35 patients with Graves' disease 25 had a LATS response above 150 % (71 % positive) (Table 1). These included 11 cases of relapsing Graves' disease, 8 of whom had a LATS titre above 200 %. These results seem to be in good agreement with most findings in the literature. A positive LATS usually ranges from 50–70 % (McKenzie 1968). A positive LATS is more frequent in relapsing Graves' disease, which is not surprising in view of the role believed to be played by LATS in the pathogenesis of hyperthyroidism in this condition. In the present small material too, the incidence of high LATS values was somewhat more marked in the cases with relapsing Graves' disease. The fact that not all patients with Graves' disease are LATS-positive has given rise to much speculation. It has been pointed out that the McKenzie assay is a rather crude method for determining small amounts of a
stimulating substance. It has also been shown that when serum from patients with Graves' disease is concentrated by various means (ammonium sulphate precipitation and chromatography of the precipitate on DEAE-cellulose or Sephadex G-200 column) LATS can be concentrated more than 10 times and that the frequency of a positive LATS may then increase to more than 80% (Munro 1967a).

Various aspects of LATS and its physiological role in Graves' disease have recently been discussed at length in a number of excellent reviews (McKenzie 1967, 1968; Munro 1967b; Lipman et al. 1967; Lemarchand-Béraud et al. 1967; Burke 1968; Ochi & de Groot 1968; Hetzel 1968).

The relationship between infiltrative exophthalmos and positive LATS has been a matter of dispute for some time; some authors think that there is a correlation, while others take the opposite view. The most recent reports indicate no correlation (McKenzie & McCullough 1968; Shillinglow & Utiger 1968; Pequengnat et al. 1967; Bonnyns et al. 1968) and in the present series (Table 2) there is no difference in positive LATS between cases with exophthalmos and those with no exophthalmos.

2) Graves' disease with nodular goitre. Four patients were classified into this category and two of them were LATS-positive with titres above 150%. This combination is more common than appears in this particular selected material. which shows, however, that Graves' disease does occur in association with nodular goitre. Plummer & Mayo (1926) expressed the view that the simultaneous occurrence of Graves' disease and endemic goitre was a coincidence. The present authors take the same view, a view which is supported by a study of the thyroid scintigrams. The scintigram of a patient with typical classical Graves' disease with diffuse thyroid enlargement is shown in Fig. 1. In Fig. 2 the scintigram of a patient with exophthalmos and nodular goitre is shown. In this case too, the uptake of radioactive iodine is of the diffuse type characteristic of Graves' disease and there is an inactive adenoma in the lower part of the left lobe. Similar findings were also reported by Selby & McClellan (1968).

One of the authors (B.-A.L.) has the impression that this coincidence is at

<table>
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<th>Group of patients</th>
<th>No. of cases</th>
<th>LATS &gt; 150%</th>
<th>LATS positive %</th>
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<td>16</td>
<td>69</td>
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<tr>
<td>Graves' diseases without exophthalmos</td>
<td>12</td>
<td>9</td>
<td>75</td>
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Table 2.

Relationship between LATS and exophthalmic syndrome in Graves' disease.
Fig. 1. Thyroid scintigram of a patient with Graves' disease.

Fig. 2. Thyroid scintigram of a patient with exophthalmos, hyperthyroidism and nodular goitre (Graves' disease with nodular goitre). In the caudal part of the left lobe there is an inactive thyroid nodule.

present less frequent than it was 10–15 years ago. This difference might be due to changes in the endemic factors which have occurred during this period (Nordman 1968; Lamberg 1968). The true incidence of such a combination is dependent on endemic conditions. This is favoured by the fact that in U. S. A. Plummer & Mayo (1926) found an incidence of 30 % nodular goitre in Graves' disease in 1926, whereas Selby & McClellan (1968) found 8 % in 1968. The findings in the next group of patients further support the impression of coincidence.

3) Toxic nodular goitre. Not many patients with toxic nodular goitre have been studied with regard to the occurrence of LATS in the blood. Werner (1963), Pinchera et al. (1965) and McKenzie (1966) did not find any LATS in the sera of patients with this type of hyperthyroidism.

On the other hand, Hoffman & Hetzel (1966) found LATS in 8 out of 16 cases with multinodular toxic goitre. Carneiro et al. (1966) had 15 cases and found no difference in positive LATS between nodular and «smooth» toxic goitre. Lemarchand-Béraud et al. (1967) had only 3 patients in this group, one of them was LATS-positive.

For this reason it is of particular interest to observe that not less than 44 % of the patients with toxic nodular goitre, which in Finland is the predominant type of hyperthyroidism, were LATS-positive. If one accepts the view, which at present seems to be fairly well founded, that the presence of LATS in the blood is an indication of Graves' disease (for the most part irrespective of the nature of the thyroid or thyroid function at the time of study), the inevitable conclusion is that at least those patients who are LATS-positive have Graves' disease in conjunction with nodular goitre. This means that in Finland at least 44 % of the patients with hyperthyroidism and nodular goitre actually have

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Graves' disease superimposed upon endemic nodular goitre. The true proportion is probably somewhat higher, since not all patients with Graves' disease are LATS-positive as assayed by the McKenzie test. Accordingly, the remainder of the patients, i.e. less than 56%, must have true toxic nodular goitre consisting of autonomous thyroid adenomata, Plummer's disease.

In principle, the LATS-positive patients are comparable to those of group 2. They have multinodular goitre but scintigrams like that shown in Fig. 2, only the parenchyma between the nodules being active. In Fig. 3 a scintigram from another patient is shown with toxic nodular goitre without any eye signs but LATS-positive. Here, it is evident that the thyroid parenchyma as a whole is activated; in addition, however, uptake of radioiodine of similar magnitude was also found in a separate adenoma which was clearly palpable in the lower part of the left lobe. In contrast to this scintigram, another is shown of a patient with a toxic nodular goitre who was LATS-negative (Fig. 4). This scintigram shows a picture typical of the classical toxic multinodular goitre, in which the parenchyma is largely inactive, but in which hyperactive and also hypoactive nodules are frequent (McKenzie 1968; Selby & McClellan 1968).

According to this, it seems logical to conclude that in Finland the patients with toxic nodular goitre fall into two groups, possibly equally large, one comprising Graves' disease (LATS-positive) superimposed upon endemic nodular goitre, and the other classical toxic multinodular goitre, with hyperactive adenomata (LATS-negative), i.e. Plummer's disease.

4) *Single toxic adenoma.* In view of the above considerations, it is not sur-

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*Fig. 3.* Thyroid scintigram of a patient with toxic nodular goitre. Evidently, both the thyroid gland as a whole as well as at least one of the nodules in the left lobe were equally hyperactive (Graves' disease with nodular goitre since the patient was LATS-positive).

*Fig. 4.* Thyroid scintigram of a patient with toxic multinodular goitre evidently of the classical type since there were hyper- and hypoactive nodules and no activation of the paranodular tissue (patient LATS-negative).
prising that in the hyperthyroid material from Finland there should be patients with a single toxic adenoma and a positive LATS assay. In the literature several scores of patients with single toxic nodules have been tested for the presence or absence of LATS in the blood and all have proved LATS-negative. Of the present series, comprising no more than 6 patients with a single toxic nodule, two were LATS-positive. One, a female patient aged 59, is still under treatment (LATS response 156 \%); the other is presented in greater detail for the sake of documentation.

The patient is a male, aged 61. According to the history, hyperthyroidism had developed gradually during the two previous years and was clinically of a low grade, but evident. The PBI was 10 µg/100 ml, the serum cholesterol 229 mg/100 ml, the triiodothyronine uptake by Sephadex 122 \%, the serum hydroxyproline 2.5 µg/ml and the urinary excretion 40 mg/m² per day, the glucose-6-phosphate dehydrogenase activity of the red cells 186 units, and the BMR +24 \%. Except for the serum cholesterol all the values were indicative of hyperthyroidism (Lamberg 1967). After these studies had been performed, the patient developed a deep venous thrombosis in the left leg and phlebography was carried out. Radioiodine studies were only made later. Three weeks after the phlebography the thyroid uptake at 24 h was 43 \% and thyroid scintigraphy showed no uptake except for a single adenoma the size of a walnut, which was easily palpable in the right lobe (Fig. 5). This rapid clearance of the iodine administered during the phlebography only 3 weeks before the scintigraphy is characteristic of hyperthyroidism. A new uptake measurement, made two months after the phlebography, now showed an uptake of 48 \%. The accumulation of radioiodine again occurred only in the nodule situated in the right lobe. From all these data (in spite of the phlebography made two months earlier and with no TSH stimulation test) and on the basis of the presence of clinically evident hyperthyroidism, the diagnosis of a single toxic adenoma was made. At this time the LATS was positive with a response of 265 \%.

The patient was treated with 9 mCi of ¹³¹I, but received no triiodothyronine at the same time, as suggested by some authors (Horst et al. 1967).

**Fig. 5.** Serial thyroid scintigrams of a LATS-positive male patient with a single toxic nodule treated with radioactive iodine. Fig. 5: Before treatment. Fig. 6: 1 year after treatment. Patient euthyroid.
One month later, a thyroid scintigram was made with the therapeutic dose showing only minimal accumulation of radioactivity in the left lobe. Treatment with triiodothyronine was still regarded as unnecessary. The size of the nodule had definitely decreased. Two months after treatment the patient appeared clinically euthyroid and three months after treatment the PBI was 5 µg/100 ml and the serum cholesterol 419 mg/100 ml. A check-up five months after treatment showed that the patient was clinically euthyroid, the PBI 6 µg/100 ml, and the serum cholesterol 350 mg/100 ml, and the thyroid scintigram showed a definite change, although there was still some hyperactivity in the right lobe. One year after treatment the patient was euthyroid, the PBI was 6 µg/100 ml, the serum cholesterol 356 mg/100 ml, the thyroid uptake 32 % in 24 h, the proportional free thyroxine 0.04 °% and the thyroid scintigram normal (Fig. 6).

The LATS response in the serum of this patient was initially over 200 °% (265 °%). Three months after treatment with radioactive iodine, LATS was still at the same level (230 °%), although the patient was now euthyroid. Two more determinations were made, seven months and one year after treatment, and in both instances LATS was no longer detectable.

How should the findings in these two cases with toxic adenoma and positive LATS in the blood be interpreted? It has been claimed that the incidence of a single toxic adenoma is not more frequent in a region with endemic goitre than in a non-endemic region (Horst et al. 1965, 1967), and, on the other hand, that the incidence of toxic multinodular goitre is more frequent in areas with slight or moderately severe endemic goitre (Clements 1960).

Hence, there may be cases with a single toxic adenoma and microscopically multinodular changes which presumably may change later on into a toxic multinodular goitre of the usual type. This matter is still an open question, since the incidence of a single toxic nodule seems to be about the same in Hamburg, Zürich and Helsinki (Horst et al. 1965, 1967; Kvist, unpubl. data). At all events, the findings in these two patients with a single toxic adenoma would indicate a coincidence of Graves' disease and a thyroid nodule. It is quite possible that the patient initially had subclinical Graves' disease and a primarily single, hyperactive but not toxic thyroid nodule (which is fairly common in endemic goitre in Finland) and that for some reason or another the nodule was more susceptible to the stimulating action of LATS than the other thyroid tissue, the result being hyperthyroidism induced in a primarily hyperactive single thyroid nodule by LATS. This is only speculation, and appears paradoxical, but the fact remains that LATS may also occur in connection with a single toxic adenoma.

In conclusion, the study carried out shows that LATS is detectable in about 70 °% of cases with Graves' disease. Furthermore, in Finland, an area of moderate or slight endemic goitre, LATS may also occur in connection with toxic nodular goitre in about half the cases. Some of these patients also have classical infiltrative exophthalmos of Graves' disease. The patients with toxic nodular
goitre can thus be divided into two groups, i.e. those with Graves' disease superimposed upon nodular goitre and those with a classical toxic multinodular goitre with hyperactive nodules, and, in addition, cases in which LATS may also occur associated with toxic adenoma.

ACKNOWLEDGMENTS

This study was aided by grants from the National Research Council for Medical Sciences, the Sigrid Jusélius Foundation and the Medicinska Understödsföreningen Liv och Hälsa.

REFERENCES

Munro D. S.: J. clin. Path. Suppl. 20 (1967a) 368.
Fond. Annual Lecture 2 (1926) 45.

Received on February 15th, 1969.