Section of Endocrinology

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Papers

Chromatographic Studies on Biopsy Specimens from Nontoxic Goitres in London Compared with Those in Thailand

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Using the methods described below, we have investigated two main groups of nontoxic goitre: (1) Subjects seen in London, a non-endemic area where the intake of iodine is not defective. (2) Subjects from a known iodine-deficient area with a high prevalence of goitre, the village of Wang Poong in north Thailand. Our main interest was to assess (*a*) whether any defect in hormone synthesis existed in the London cases of sporadic goitre, in whom the intake of iodine was found to be normal; and (*b*) whether such a defect was also to be seen in the iodine-deficiency goitres of the Thai endemic area.

Before presenting these biopsy studies of intrathyroidal iodine metabolism comparing the London sporadic and Thai endemic goitres, we briefly review the basic steps in the biosynthesis of thyroid hormone in the normal thyroid.

Biosynthesis of Thyroid Hormone

Inorganic iodide is trapped by the thyroid cell from the plasma and oxidized to elemental iodine by an oxidative system. The iodine immediately attaches itself on tyrosyl residues present in peptide linkage in thyroglobulin, to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). Two iodotyrosine molecules are coupled to form iodothyronines (ITH), thyroxine (T_4) and triiodothyronine (T_3). All these iodoaminoacids are released from their peptide linkage in thyroglobulin under the action of a protease. The free iodothyronines enter the circulation, but no free iodotyrosines can escape, as they are immediately deiodinated by a specific deiodinase, which enables the liberated iodine to re-enter the cycle.

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Our procedure for studying this intrathyroidal iodine transfer consisted of sampling the thyroid by a drill biopsy needle at a standard time after a tracer dose of radioactive iodine (48 hours after 100 μc ^{131}I Na). This labelled thyroid tissue was then digested and chromatographed to separate the individual iodocompounds, i.e. the inorganic iodide, the two thyroid hormone precursors, mono- and diiodotyrosine, and the fully formed thyroid hormones, the iodothyronines, thyroxine and triiodothyronine.

The distribution of both radioactive (^{131}I) and stable (^{127}I) iodine among these iodocompounds was measured, which provided indices of: (*a*) The speed of thyroid hormone formation (from ^{131}I measurements). (*b*) The size of the thyroid's hormone stores (from ^{127}I measurements).

Methods for Digestion of Thyroid Tissue, Chromatography and Iodimetry

Most digestion methods employ enzymic hydrolysis to liberate the iodoamino acids from their peptide linkage in thyroglobulin. In our technique, the samples were homogenized in veronal buffer pH 8·5 immediately after collection and digested with a mixture of pancreatin, erepsin (1 mg of each enzyme for 10 mg of tissue) and hyaluronidase (500 i.u.) at 37° C with shaking for 36 hours. This method was found to give full and safe digestion within 24 hours, while other enzymes either needed longer times or degraded thyroxine. Under the above conditions no deiodination of thyroxine occurred, and the percentage of iodide present in the digest was not higher than that present in the undigested homogenate.

The digests were then purified on ion exchange resin columns. The organic iodocompounds were eluted from the resins and fractionated further by paper chromatography in an n-butanol – $2N.CH_3.COOH$ ascending solvent system. The samples were always chromatographed in duplicate; to the second spot standard solutions of KI, MIT, DIT and T_4 were added, to ensure identity of the spots. Fractions of the digest and organic eluate were used to estimate the total and organic stable iodine by a dry alkaline ashing technique. The paper chromatograms were first scanned for radioactivity distribution in our automatic Geiger-Müller scanning device (with two Geiger-Müller tubes with thin mica windows, shielded with 2 in. lead, and found to have a sensitivity for ^{131}I

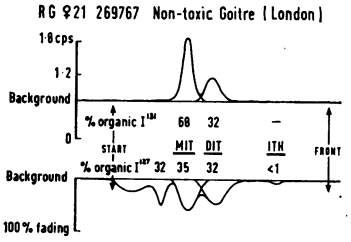

Fig 1A ^{131}I *and* ^{127}I *scannings of a chromatogram of resin-purified thyroid tissue digest from a London sporadic goitre (young diffuse). Note high 131-MIT/131-DIT ratio and minimal iodothyronines in both* ^{131}I *and* ^{127}I

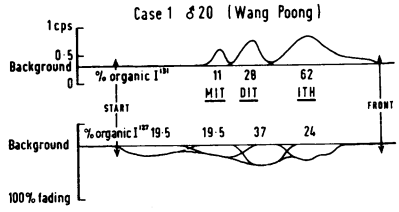

Fig 1B ^{131}I *and* ^{127}I *scannings of a chromatogram of resin-purified thyroid tissue digest from a young Thai subject with diffuse endemic goitre. Note high percentage of* ^{131}I *as iodothyronines and low 131-MIT/131-DIT ratio*

of 10^{-4} μc/sq.cm for twice background). After counting, the paper strips were stained to measure chemically the distribution of stable iodine on the same chromatogram. The paper was sprayed with the ceric arsenite reagent and scanned in a specially designed densitometer. The iodocompounds appear as white spots on a yellow background, so that the negative peaks on the densitometry record are measured by planimetry (Dimitriadou & Fraser 1964). This method detects 6×10^{-9} g ^{127}I/spot and measures over the range of 0·01 to 0·8 μg I.

RESULTS

Sporadic (London) Goitres

The London group consisted of 17 female subjects aged 13 to 70 years, chosen from patients presenting at Hammersmith Hospital as examples of nontoxic goitres found to have high normal or avid uptakes of ^{131}I (i.e. >35% at 48 hours). The goitre sizes varied from 35 to over 200 g; 9 of these were diffuse and 8 nodular. In all cases the diagnosis was confirmed by histology. Fig 1A is an example of the chromatography results from the thyroid digest in these sporadic nontoxic goitres. This case was a young patient with a diffuse goitre, but similar results were also obtained in most sporadic nontoxic goitres studied (*see* Fig 2). Most of the gland's radioiodine (Fig 1A) was in the MIT area, and there was no discernible radioactivity over the iodothyronine area. Similarly, most of the stable organic iodine (^{127}I) was found stored in the gland in the form of precursors only. In a normal gland at 48 hours after ^{131}I administration, there is always less radioactivity in the MIT than in the DIT fraction, and approximately 20% of it is present as iodothyronines. The stable organic iodine is, on the other hand, uniformly distributed between the two precursors and the iodothyronines. Thus, these findings show that in sporadic nontoxic goitre there is a delay in the transfer of iodine to the fully formed hormone stage, and as a consequence the stores of fully formed hormone are low. Fig 2 summarizes the ^{131}I findings in all sporadic nontoxic goitres studied, along with findings in some thyrotoxics and normals for comparison. The 8 subjects forming our control group were 7 London patients undergoing neck operations for other reasons (1 for melanoma of the palate, 4 for hyperparathyroidism, 1 for carcinoma and 1 for Riedel's thyroiditis restricted to other parts of the gland) and one thyrotoxic subject biopsied when euthyroid. As not all of them were clearly normal, they have been called 'probable normals'. In the nontoxic goitre group the percentage of radioactivity present as MIT is higher and the percentage present as iodothyronines lower than in the normals and also the thyrotoxics, although these latter had a rapid turnover, as indicated by the high PB ^{131}I values. Only two of the nontoxic goitres had a PB ^{131}I value above the normal

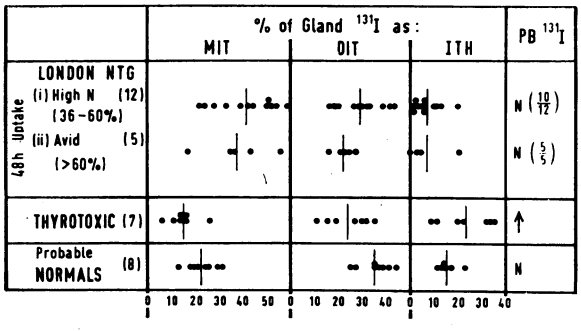

Fig 2 *Collected data from thyroid tissue digest chromatography in all London sporadic nontoxic goitres studied (NTG), 7 thyrotoxics and 8 normals, showing percentage distribution of* ^{131}I *as MIT, DIT and iodothyronines, and whether PB* ^{131}I *is normal or high*

range, and in both cases the radioactivity was butanol insoluble, i.e. not T_4. In none of these nontoxic goitres studied, whether young or old, diffuse or nodular with high normal or avid uptake, did we find a rapid transfer of ^{131}I as should be expected in simple iodine deficiency. The gland's radioactivity present as iodothyronines was never over 20%, and in most of the cases (12/17) well below our lower normal limit.

Fig 3 summarizes the ^{127}I findings in the same groups, i.e. the concentration of stable iodine and its percentage distribution as MIT, DIT or iodothyronines. All goitres showed the very familiar abnormality of a low iodine (^{127}I) concentration. A similar finding in the thyrotoxics doubtless depends on their rapid turnover. Further, while the normals show that the gland's iodine (^{127}I) is distributed approximately equally between the three iodocompounds, the nontoxic goitres seem to be particularly depleted in their iodothyronine content. Thus, these ^{127}I findings confirm the ^{131}I results. Similar abnormalities were also seen in 2 cases of sporadic nontoxic goitres investigated after iodine repletion as described by Burrell & Fraser (1957). Thus, in these sporadic nontoxic goitres, thyroid hormone is formed more slowly than in normals, and presumably as a consequence the gland stores of fully formed hormone are low, which implies a defect in the gland's mechanism for thyroxine synthesis.

In these sporadic nontoxic goitres, who were all euthyroid, the defect is probably a minor failure, and adequately compensated by the goitre formation, i.e. enough thyroxine is made to keep the subjects euthyroid, but without any surplus for storage. Similar results to ours have been reported by Beckers and his collaborators for the sporadic nontoxic goitres studied in Belgium (Beckers 1962), and also by Pitt-Rivers *et al.* (1957) and Trunnel & Wade (1955) for some euthyroid nodular goitres. Whether this defect is an intrinsic thyroid abnormality or the result of ingestion of some goitrogenic substance cannot be judged from these studies, for some known goitrogens can cause similar defects in intrathyroidal iodine transfer. We have had the opportunity to investigate a patient who was treated with phenylbutazone for severe rheumatoid arthritis, and who developed a goitre five years after treatment was started (200 mg phenylbutazone a day). While still receiving the drug (ten years after therapy started and five years after the large goitre had been noticed), the biopsy from her large nodular gland showed on chromatography similar findings to those seen in the sporadic nontoxic goitres.

Endemic Area Goitres
(studied in the village of Wang Poong in the Thai hills)

As the conditions in the tropical laboratory were different from those in London, it was important to test if the methods were working adequately under field conditions. For this purpose, we first analysed labelled glands from some rabbits and also made biopsy studies on two thyrotoxic subjects from a town near the endemic village. In both instances the results obtained confirmed the adequacy of the methods, and so the validity of comparisons with the London data.

From the inhabitants of the endemic village, 3 young subjects with small diffuse goitres were chosen for biopsy studies. Also, later from this area the glands from 2 elderly subjects with large nodular goitres were analysed similarly, when they had their huge goitres excised surgically. For it seemed possible that these larger nodular goitres, seen occasionally in the older subjects in endemic areas, depended on some other factor in addition to iodine deficiency.

The findings seen in all 3 young Thai subjects with the iodine deficient goitres are illustrated in Fig 1B. In contrast to the sporadic goitres here, most of the gland's radioiodine is in the form of iodothyronines and least as MIT – resembling more the thyrotoxic. The stable iodine, although very low, is normally distributed between the three iodocompounds. Here, therefore, there is no sign of a defective hormone synthesis, but instead evidence of an overactive synthetic mechanism to compensate for iodine deficiency. However, this pattern was not seen in the older nodular endemic goitres, where the distribution was strikingly similar to that found in the sporadic goitres. A probable explanation for this might be that pro-

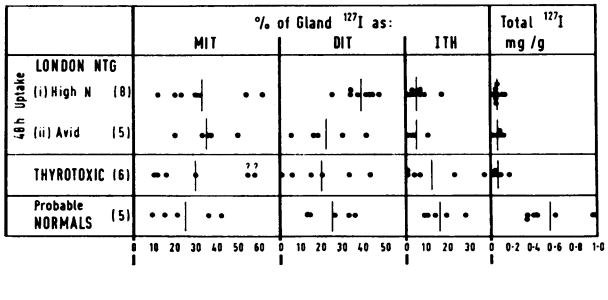

Fig 3 *Collected data from thyroid tissue digest chromatography in all London sporadic nontoxic goitres studied (NTG), thyrotoxics and normals showing total stable iodine concentration and its percentage distribution as MIT, DIT and iodothyronines*

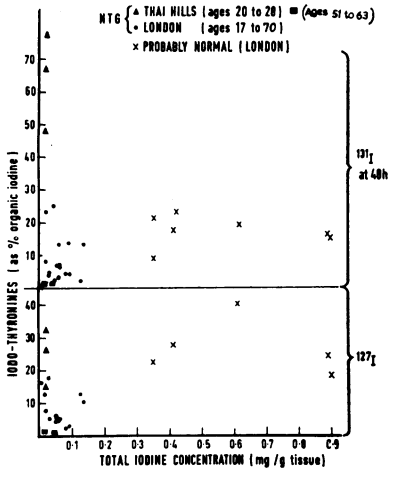

Fig 4 *Summary of biopsy findings comparing 3 diffuse endemic goitres in young Thai subjects with 2 nodular endemic goitres in older Thai subjects and 17 London sporadic goitres. Note low total iodine concentration in all goitres, and high percentage as iodothyronines only in the young Thai endemic goitres*

longed overactivity – as seen in the young persons – may eventually lead to exhaustion and failure of the synthetic mechanism. Others (De Visscher *et al.* 1961), who have investigated the intrathyroidal iodine metabolism in the Congo endemic area, have reported findings similar to our results in the large nodular goitres only, but these authors have studied the iodocompounds in some of the goitres removed surgically – clearly any surgical excision would be offered only to the larger goitres.

Finally, Fig 4 summarizes our findings by contrasting the various groups in their two major biopsy features, the ^{127}I concentration as mg I/g tissue and the percentage of the gland's organic iodine present as iodothyronines. All types of goitre have a low iodine concentration, but the young Thai endemic goitres seem to be able to utilize this scarce iodine more effectively than the normals – transferring it rapidly and incorporating a normal proportion into fully formed thyroid hormone. In contrast to this, the older nodular endemic goitres and all the sporadic goitres studied are defective in this last respect.

In summary, in all sporadic nontoxic goitres studied, whether young or old, diffuse or nodular, we have found a defective hormone synthesis which may be the cause of goitre. In contrast to this, in the iodine-deficient area studied no such abnormality was found in the young subjects, while the older subjects with huge nodular goitres do show the same defect as the sporadic goitres – possibly because their overactive glands have failed.

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Pitt-Rivers R, Hubble D & Hoather W H (1957) *J. clin. Endocrin.* **17**, 1313
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The following paper was also read:

Clinical Studies of Nontoxic Goitres in London Compared with Those in an Iodine-deficient Area in Thailand

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