THE FORMATION OF THYROGLOBULIN
IN HUMAN THYROID MEDULLARY CARCINOMA

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ABSTRACT

The biosynthesis of thyroglobulin has been investigated in five well-demarcated thyroid medullary carcinomas. The biochemical methods used allowed the study to be performed on small amounts of tissue, about 2–4 mg. The biochemical results were compared with the morphological structure.

A normal biosynthesis was obtained in three of the five investigated tumours. No indication of a biosynthesis was obtained in the remaining two tumours. The morphological investigation revealed that normal thyroid follicles were found in the tumour tissue close to the pieces with a normal biosynthesis. Normal thyroid follicles could not be found in the tumour tissue with no sign of biosynthesis. Metastases from two medullary carcinomas were also investigated. No indication for a biosynthesis of thyroxine or thyroglobulin was found. Normal thyroid follicles could not be demonstrated.

It is concluded that the biosynthesis of thyroglobulin in well-demarcated medullary carcinomas occurs in normal thyroid tissue present within the tumour. It is also concluded that all studies on the function of thyroid carcinomas must involve studies on very small pieces of fresh tissue in order to avoid heterogeneity and must involve a morphological examination of the surrounding tissue.
Preliminary results have indicated that follicular and mixed follicular-papillary carcinomas synthesize thyroglobulin as judged by the in vitro incorporation of radio-iodide and 3H-leucine into 17–19 S proteins and identified as thyroglobulin by sucrose gradient centrifugation, ammonium sulphate precipitation and disc gel electrophoresis. No biosynthesis of thyroglobulin was detected in papillary or anaplastic carcinomas under similar conditions. It was also found that 17–19 S thyroglobulin was synthesized in well-demarcated medullary carcinomas (Ljunggren et al. 1971).

The formations of thyroglobulin in medullary carcinomas was rather unexpected as the tumour does not contain cells likely to synthesize this protein.

The purpose of the present investigation was to elucidate which cells in the medullary carcinoma are responsible for the biosynthesis of thyroglobulin.

The results indicate that the biosynthesis of thyroglobulin in well-demarcated medullary carcinomas occurs in normal thyroid follicular epithelium present within the tumour.

METHODS

Thyroid tissue was obtained at surgery and pieces of about 20 mg each were cut out from different parts. The central part of each piece was analyzed biochemically and the surrounding tissue of each piece was analyzed morphologically.

The biochemical analysis was performed immediately after the operation and has recently been described in detail (Ljunggren et al. 1971). Specimens of about 2-4 mg were incubated in Eagle’s medium (without 1-leucine) in the presence of 3H-leucine and 125I and analyzed after salt extraction and sucrose gradient centrifugation. Pieces of about 0.5 g each of macroscopically homogeneous tissue were also analyzed by ammonium sulphate precipitation after salt extraction in order to investigate further the presence of thyroglobulin.

Five patients with thyroid medullary carcinoma were investigated. Between 5 and 10 different pieces from each tumour were analyzed and normal thyroid tissue was used as control in all experiments. Two metastases from two medullary carcinomas were included. The biochemical and morphological investigation were made separately and the results were not compared until the whole investigation was completed.

RESULTS

No indication of a biosynthesis of thyroglobulin could be obtained in two of the five investigated medullary carcinomas. A typical example of the in vitro incubation is presented in Fig. 1 together with the results from a normal gland. The peak around fraction number 33 represents 17–19 S proteins as compared to purified adult human thyroglobulin. The peak around fraction number 8 represents slower sedimenting components corresponding to 3–8 S proteins.
Sucrose gradient centrifugation analysis of the soluble proteins from a medullary carcinoma (top) and normal thyroid tissue (bottom). Tissue specimens were incubated for 4 h at 37°C in the presence of $^3$H-leucine and $^{125}$I in Eagle's medium (lacking leucine). A salt extract (0.1 M KCl - 0.02 M phosphate buffer, pH 7.4) of the incubation mixture was dialyzed and analyzed by sucrose gradient centrifugation. Linear sucrose gradient of 5 to 20 per cent (w/v) was used in the SW50-rotor of the Spinco model L centrifuge. Time of centrifugation 225 min at 200 000 × g. Sedimentation is shown from left to right.

It can be seen that normal tissue incorporated both $^3$H and $^{125}$I in the 17–19 S region whereas no protein or incorporation in these region can be demonstrated in the tumour tissue. No precipitation was obtained when the tumour tissue was fractionated between 1.2–1.8 M ammonium sulphate concentration. Thus, the formation of thyroglobulin or of thyroxine could not be demonstrated.

However, a normal distribution pattern of thyroglobulin was obtained from three of the five investigated carcinomas. Both protein, $^3$H- and $^{125}$I-activity was demonstrated in the 17–19 S region after the ammonium sulphate fractionation. A typical example was demonstrated in the preliminary report (Ljunggren et al. 1971).

The biochemical results were compared with those from the morphological investigations. These results revealed that colloid containing thyroid follicles were found in the tumour tissue close to the pieces taken for the biochemical
Medullary carcinoma. Two tissue sections from different parts of the same tumour. H. E. × 190.

Top: Uniform carcinoma cells and various sized masses of hyaline material which stained positively for amyloid.

Bottom: Non-neoplastic colloid containing thyroid follicles surrounded by sheets of carcinoma cells.
analysis in all tumours demonstrating the presence of 17–19 S proteins. Thyroid follicles could not be demonstrated in the tumour tissue which biochemically showed no signs of 17–19 S proteins.

Tissue sections from a typical case of incorporated non-neoplastic thyroid follicles within a medullary carcinoma containing 17–19 S proteins are shown in Fig. 2.

No indication for a biosynthesis of thyroxine or thyroglobulin could be obtained in the metastases. Nor could any normal thyroid follicles be found.

The results, thus, indicate that the biosynthesis of thyroglobulin in medullary carcinomas most probably occurs in normal thyroid tissue present within well-demarcated medullary carcinomas.

DISCUSSION

The formation of thyroglobulin in human thyroid tumours has been studied in several investigations during recent years (Pochin et al. 1965; Ramagopal et al. 1965; Lupulescu et al. 1968; Valenta et al. 1968; Thomson & Bissett 1969; Valenta & Lemarchand-Béraud 1970; Ljunggren et al. 1971). The results have indicated that thyroid tumours can contain thyroglobulin despite the absence of radio-iodide uptake on scintiscanning in vivo. However, the absence of uptake can only be demonstrated in these cases if normal tissue is present. Thus, the tumour tissue which contains thyroglobulin has a decreased uptake of radio-iodide in relation to normal tissue. The amount of thyroglobulin and the degree of iodination in the thyroglobulin containing tumours was lower than in normal thyroids. However, the ability of thyroglobulin from tumour tissue to be iodinated is not impaired as compared with the thyroglobulin from normal tissue. In addition to 19 S thyroglobulin, lighter components in the range of 4 S were found in tumour tissue and in relatively higher amounts as compared to the normal. There has as yet been no evidence for the presence of an abnormal thyroglobulin in human tumour tissue with regard to a pathological pathway of biosynthesis or to the chemical structure of the protein. Some investigators have found an increased amount of 12 S components or the presence of iodinated proteins with a sedimentation coefficient lower than the normal 19 S. However, it is likely that these substances are caused by the use of frozen tissue. It is now well established that 17–19 S thyroglobulin is unstable at low temperatures, especially when poorly iodinated.

The above mentioned results are mainly based on the use of a fairly large amount of tumour tissue, often more than 0.5 g in weight and often previously frozen. It is, thus, difficult to ascertain which structure is producing a certain substance. The main difficulties in the evaluation of the biosynthesis of thyroglobulin and the thyroid hormones in the carcinomas are caused by the hetero-
geneity of the tissue. A compromise must thus be found between the desire to use such a small amount of tissue that the homogeneity can be ascertained or to obtain such a large amount of substance that it would allow a proper chemical identification.

The present investigation illustrates these difficulties. Even if such a small amount of tissue as 2–4 mg is taken from a fresh, well-demarcated and macroscopically homogeneous medullary carcinoma the results demonstrate that the formation of thyroglobulin found most likely derives from the presence of scattered areas of normal tissue within the tumour. Other types of thyroid carcinomas are now under similar investigation.

The production of calcitonin by medullary carcinomas is now well established and the results have been reviewed (e.g. Kaplan & Peskin 1971; Keynes & Till 1971). The results obtained here have not been further analyzed in this investigation.

ACKNOWLEDGMENTS

The study was supported by grants from the Swedish Medical Research Council (projects No. B72-19X-2442-05A, B73-19X-2442-06B), Karolinska Institutets fonder and Svenska livförsäkringsbolags nämnd för medicinsk forskning.

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Received on December 21st, 1972.