Chapter IV

HISTOLOGY OF JUVENILE THYROID GLAND
INTRODUCTION

Anatomists of the fifteenth and sixteenth centuries described the thyroid gland in detail, and an enlargement of the thyroid as the anatomical basis for goiter was subsequently noted (Lason, 1946). In 1895 Magnus-Levey demonstrated that the feeding of dried animal thyroids to normal men increased their metabolic rate. This suggested that the thyroid gland might contain a substance that affected the cellular activity of other organs. In 1895 Kocher of Bern demonstrated the high amount of iodine in a thyroid concentrate.

The thyroid gland is the site of synthesis, storage, and controlled secretion of thyroid hormones. Thyroid hormones are synthesized by the iodination and coupling of a small subset of tyrosyl residues within the peptide chains of thyroglobulin (Tg). The functional components of the thyroid gland are the individual thyroid follicles, which consist of a cuboidal epithelium arranged as a single layer surrounding a lumen that contains a colloid material. The follicular cells synthesize a protein, thyroglobulin, which is released into the colloid space by vesicular exocytosis. Thyroglobulin is important as substrate for tyrosine iodination and the subsequent synthesis of thyroid hormones. Iodide is converted by a peroxidase at the luminal surface of the cell, to an oxidised species of iodine that is incorporated into the tyrosyl groups of thyroglobulin as monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues. Within the Tg, iodinated tyrosines undergo an oxidative coupling that results mainly in the formation of T4 and smaller amounts of T3. This oxidative coupling is catalysed by peroxidase which is also responsible for the conversion of iodide to iodine. (Silva, 1988).

Thyroid hormones are unique in that they are complexed through covalent bonds to iodine. The thyroid follicular cells are able to trap iodide at the base of the cell and transport it against an electrical gradient across the cell. (Golstein, 1992). In response to endocrine
stimulation follicular cells engulf the colloid by phagocytosis. The colloid within the endocytotic vesicles is enzymatically degraded to yield thyroid hormones that are released from the follicular cells into the extracellular space. For the newly formed hormones to reach the bloodstream, Tg is reabsorbed and degraded by the follicular cells. Hypothalmic cells 'sense' a decrease in the serum levels of thyroid hormones and secrete thyrotropin releasing factor (TRH), a peptide that prompts specific cells in the anterior pituitary gland to secrete thyroid stimulating hormone (TSH). Continued stimulation of the thyroid by TSH results in a great increase in the quantity and activity of the synthetic machinery of the follicular cells (Vassart, 1992). The cells become columnar in shape and the luminal content of the colloid is greatly decreased. Certain drugs inhibit thyroid function by antagonizing formation of thyroid hormones. These drugs are generally classified into those compounds that inhibit iodide transport (iodide trapping) into the thyroid gland and those that inhibit iodine incorporation into tyrosine. Iodide in large doses is transiently inhibitory to thyroid function (Wolff-Chaikoff effect), but the mechanism of this inhibition remains unexplained. Iodide pump inhibitors probably antagonise iodide transport through competitive inhibition. Maintenance of an effective concentration of antithyroid drugs for a sufficient time causes thyroid hypertrophy and goiter (Wolff, 1989). Failure of the thyroid to produce T4 and T3, and in the absence of thyroid hormone production TSH stimulation continues to lead to hypertrophy of the thyroid follicular cells. Thyroid glands from such hypothyroid individuals are hyperplastic and exhibit columnar follicular cells with a decreased amount of colloid.

The essential role of the thyroid glands in the control of numerous physiological process is now well documented. Thyroid hormones influence most bodily functions (Green, 1987). They directly affect a number of physiological processes and, although without observable actions, they are often required as they exert effects within almost every tissue of the body throughout the life of an individual.
Materials and Methods

A. Animals for study and their maintenance:

Juvenile male rats (body wt - 40-50 g) were used for this study and maintained as described in Chapter III.

B. Materials for injection and treatment:

i) Phenylhydrazine hydrochloride (PHH):

As described in Chapter I.

ii) L-thyroxine-Sodium Salt (T4):

As described in Chapter I.

C. Reagents for histological study:

i) 10% Neutral Formaldehyde:

a) 10% Calcium Chloride (CaCl₂):

Calcium chloride was a product of E. MERCK, India. 10 g of CaCl₂ was dissolved in a total volume of 100 ml with double distilled water.

b) 10% Neutral Formaldehyde:

Formaldehyde solution (AR) was a product of S.d. Fine-Chem Pvt. Ltd., India. A 10% neutral formaldehyde solution was used for fixing thyroid gland. Its composition is given below:

- 40% formaldehyde - 100 ml
- Double Distilled water - 500 ml
- 10% Calcium chloride - 100 ml

Add double distilled water to make 1000 ml and add marble chips.
ii) **Paraffin**:

Paraffin (m.p - 58-60°C) was a product of E. MERCK, India.

iii) **Xylene**:

Xylene was also a product of E. MERCK, India.

iv) **Xylol - paraffin mixture**:

Xylol, was saturated with paraffin, m.p. 58-60°C. The fixed tissues after dehydration and clearing were kept in this mixture for 30 minutes just before embedding in paraffin.

v) **Alcohol**:

Absolute alcohol was a product of Bengal Chemical and Pharmaceutical Works Ltd., India. Graded alcohols 30%, 50%, 70%, 90%, 95% and absolute alcohol were used for dehydration. Graded alcohol were obtained by diluting absolute alcohol with double distilled water.

vi) **Hematoxylin (Ehrlich's Hematoxylin)**:

Hematoxylin Crystals

(G.T. Gurr Ltd., London) - 2 g

Ammonia alum

$\text{Al}_2(\text{SO}_4)_3 (\text{NH}_4)_2\text{SO}_4.24\text{H}_2\text{O}$ - 3.0 g

Alcohol - 100 ml

Glycerol - 100 ml

Double Distilled water - 100 ml

This solution ripens in 6-8 weeks and subsequently used for staining.
vii) *Eosin*:

Eosin - 1.0 gm  
(E. MERCK AG. Darmstadt)

70% ethyl alcohol - 100 ml

Glacial acetic acid - 5 ml

This solution was diluted with equal volume of 70% alcohol, and 2-3 drops of acetic acid was used just prior to staining.

viii) *DPX mountant*:

DPX, a product of Loba Chemie Pvt. Ltd., India.

ix) *Mayer’s Albumin fixative*:

Mayer’s albumin fixative was prepared by beating the white of an egg only until well broken up, but not stiff. The solution was poured in a cylinder to remove the air and the liquid from the bottom was added to an equal volume of glycerol. A bit of thymol (1:100) was added to prevent the growth of moulds. This solution was finally filtered through glass wool and refrigerated.

**D. Fixation of thyroid gland and paraffin embedding**:

The thyroid glands were surgically removed from the juvenile rats under light ether anaesthesia. The glands were gently blotted over blotting paper and fixed in 10% neutral formaldehyde for 48 hrs. The tissues were dehydrated by passing through graded alcohols (30%, 50%, 70%) for 30 minutes each. The tissues were kept in 95% alcohol for 20 minutes and finally dehydrated with absolute alcohol for 30 minutes. The tissues were cleared off alcohol by passing them through xylene for 10 minutes. Finally the whole thyroid mass was soaked in saturated xylol-paraffin mixture and dipped into molten paraffin in embedding bath. The tissues were embedded for one hr. at 58-60°C under vacuum. Subsequently uniform paraffin blocks of the thyroid glands were made.
**E. Sectioning:**

5-6 μ thick sections were cut with a microtome manufactured by American Optical Company, USA. The sections were adequately stretched by first floating the ribbons over water on slide (with Mayer's albumin) and finally placing the slides on hot plate.

**F. Histological staining:**

1. The thyroid sections were deparaffinized by keeping them dipped in xylene for 10 min and further cleared the paraffin by keeping the slides in fresh xylene for another 10 minutes.

2. Xylene was removed by passing the slides through graded alcohol concentration in the order of absolute alcohol, 90%, 70%, 50% and 30% alcohol for 10 minutes.

3. Sections of thyroid gland was then washed in double distilled water and kept in hematoxylin stain for 10 minutes.

4. The sections were then washed in running water for 5 minutes.

5. The sections were passed through descending grades of alcohol (30%, 50%, 70%) for 10 minutes each.

6. The sections were further subjected to Eosine staining for 5 minutes.

7. Removal of excess Eosine was performed by passing the slides through absolute alcohol.

8. Alcohol from the sections was removed by dipping the sections in xylene for 20 minutes and finally mounted in DPX. Photographs of the sections were taken using a microscope (Leitz, DIAPLAN, Made in Germany) with photomicrographic attachment at magnification: x240.
Results

The sections of thyroid gland prepared from the control animals showed typical follicular structures, consisted of cuboidal epithelium arranged in thin layers surrounding a lumen that contained colloid materials. Some follicles appeared to contain intact colloids and some to contain partially resorbed colloid materials.

Injections of different doses of PHH (10, 20 and 30 μg per g) to the juvenile rats caused alterations in the histological structure of the thyroid gland in a dose-dependent manner. PHH caused more resorption of the colloid materials and disruption of the cuboidal epithelial cell layers. At 10 μg/g of PHH, resorption of the colloid material associated with hyperplastic nature of the epithelial cells. The gland took a hypothyroid appearance. Higher dose of PHH (20 μg/g) injections effected to intensify hypothyroid appearance of the gland. More resorption of colloidal material and more intense hyperplastic appearance of the epithelial cells in many affected areas have been found. Hypertrophy of the epithelial cells were not much prominent. Injections of still higher dose (30 μg/g) of PHH made the thyroid gland to get a typical goitrous appearance, having practically a few follicles, others being resorbed and invaded by the hyperplastic epithelial cells, on the day 3, after the drug injection. The gland histologically appeared to be a packed mass of hyperplastic epithelial cells.

Injection of different doses of T4 (1, 2, 4 and 8 μg/g) to normal juvenile rats also demonstrated an appearance of the gland consisting of normal follicles made of colloids and cuboidal epithelial cells as observed on the 3rd day. Dose-dependent effect of T4 was less prominent. Appearance of vacuolated space in or around some follicles in the T4-treated thyroids, might be due to artifacts occurred during preparation of the sections and subsequent staining.

Interesting changes in the histological appearance of the thyroid was observed when T4 (1, 2, 4 and 8 μg per g) was injected in combination with PHH (20 μg/g).
T₄ at 1, 2, 4 and 8 μg/g doses with PHH (20 μg/g) mostly protected the thyroid gland from
the toxic effect of PHH (20 μg/g), when compared with the only PHH (20 μg/g) injected
animals. In the T₄ (1-8 μg/g) plus PHH (20 μg/g) treated animals the sections of the thyroid
gland contained more intact follicles than the only PHH (20 μg/g)-treated gland, as
observed on the 3rd day. Injection of T₄ (1 μg/g) with PHH (20 μg/g) was found to be
inadequate to maintain the normal histological structure as the sections contained
numerous layers of thyroid epithelial cells in the interfollicular spaces. T₄ at 2 μg/g with PHH
(20 μg/g) dose was found to be optimum for maintenance of the mostly normal thyroid
follicles on the day 3. However, injections of higher doses of T₄ (4 and 8 μg/g) with PHH (20
μg/g) again showed appearance of thick layers of cuboidal cells in the interfollicular space.
Plate 1. Effect of different doses (10, 20 and 30 μg per g) of PHH injections on the histological structure of thyroid gland in juvenile rat. A. CONTROL. B. PHH (10μg/g) C. PHH (20μg/g) D. PHH (30μg/g). Note the goitrous appearance of the thyroid gland of various degree: x240

Plate 2. Effect of different doses (1, 2, 4 and 8 μg per g) of T₄ injections on the histological structure of the thyroid gland in juvenile rat. A. CONTROL. B. T₄ (1μg/g) C. T₄ (2μg/g) D. T₄ (4μg/g) E. T₄ (8μg/g): x240

Plate 3. Effect of injections of PHH (20 μg/g) and PHH (20 μg/g) plus T₄ (1, 2, 4 and 8 μg per g) on histological structure of thyroid gland in juvenile rat. A. CONTROL. B. PHH (20 μg/g) C. PHH (20 μg/g) + T₄ (1 μg/g) D. PHH (20 μg/g) + T₄ (2 μg/g) E. PHH (20 μg/g) + T₄ (4 μg/g) F. PHH (20 μg/g) + T₄ (8μg/g). Note the counteraction of the effects of PHH by T₄ at various degrees: x240