Thyroid problems in pediatric oncology: damage, prevention and consequences
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Preface

The prognosis for many childhood malignancies is improving. It has been estimated that, at this moment, one out of every 750-800 young adults is a survivor of childhood cancer. Consequently, (late) side effects of given treatments are becoming more apparent and more relevant. One of the most common sequelae of cancer treatment is damage to the endocrine system. The hormones produced by this system, especially thyroid hormone, are of major importance for optimal growth and development into adolescence.

The observed endocrine disruptions may be the result of several factors, related to the tumor, the chemo- and/or the radiation therapy, which can all act additive or even synergistic. For example, growth retardation in a child following cancer treatment may be caused by radiation-damage to the long bones and the spine, pituitary dysfunction (GH and/or TSH deficiency) due to craniospinal irradiation, and low levels of IGF-1 or TSH due to a bad nutritional state or the administration of cytotoxic drugs. For this reason, it is important to be aware of the whole spectrum of endocrine effects that may be seen after cancer treatment.

Thyroid (axis) damage due to cancer in childhood may occur after treatment for neuroblastoma (NB), Hodgkin's disease, acute lymphoblastic leukemia (ALL), rhabdomyosarcoma, naso-pharynx carcinoma and brain tumors. The tumor or the treatment may cause structural or functional damage to the hypothalamus, the pituitary gland or the thyroid, the latter expressed as (subclinical) hypothyroidism or even hyperthyroidism. Pituitary or hypothalamic thyroid axis dysfunction is mostly expressed as hypothyroidism. Structural abnormalities of the thyroid are often benign but thyroid cancer is a frequently reported late effect of radiotherapy.

In this chapter, the field of pediatric endocrinology, with special attention to the thyroid-pituitary-hypothalamic axis, the field of pediatric oncology, with special attention to neuroblastoma and thyroid tumors and finally the consequences of treatment for childhood cancer on the endocrine system are discussed, resulting in the outline of this thesis.
1.1 The thyroid system
1.1.a. Endocrine glands

The endocrine glands involved in this thesis are the hypothalamus, the pituitary gland and their peripheral target organs. The hypothalamus is a vital link between the various regions in the brain and the six main anterior pituitary hormones. Most hormones are stimulated through their own “axis”, which have a “negative feedback mechanism”, meaning that high concentrations of the end products negatively influence the concentration of the hormones secreted by the pituitary and hypothalamus.

The thyrotropic axis consists of thyrotropin-releasing hormone (TRH), released by the hypothalamus, which stimulates the pituitary gland to secrete thyroid stimulating hormone (TSH, also called thyrotropin), to stimulate the thyroid gland in producing and secreting the thyroid hormones thyroxine (T₄) and in minor amounts tri-iodothyronine (T₃). The corticotropic axis consists of corticotropin-releasing hormone (CRH) produced by the hypothalamus, followed by adreno-corticotropic hormone (ACTH, also called corticotropin) produced by the pituitary gland and subsequently the glucocorticoids and androgens by the adrenal cortex. The posterior pituitary hormone vasopressin may also stimulate ACTH secretion.

The gonadotropic axis produces testosterone (males) or estrogens and progesterone (females) from the gonads after stimulation by luteinising hormone (LH, also called lutropin) and follicle stimulating hormone (FSH, also called follitropin) from the pituitary gland and gonadotropin-releasing hormone (GnRH) from the hypothalamus. The somatotropic axis produces several growth factors. The main circulating growth factor is insulin-like growth factor 1 (IGF-1), mainly secreted by the liver after stimulation by growth hormone (GH, also called somatotropin) produced by the pituitary gland and growth hormone-releasing hormone (GHRH) from the hypothalamus. The production of GH is inhibited by the hypothalamic hormone somatostatin.

Lastly, the lactotropic cells in the anterior pituitary gland produce prolactin (PRL) that elicits lactation. Its production is controlled by dopamine and TRH secreted by the hypothalamus.

The posterior pituitary gland secretes two hormones, vasopressin (anti-diuretic hormone, ADH) and oxytocin, which are synthesized in the hypothalamus and
subsequently stored in the pituitary. ADH is responsible for maintaining a normal plasma osmolality and oxytocin is required during labor (uterus contractions) and lactation. This thesis is targeted to the thyroid axis.

1.1.b. The thyroid gland & thyroid hormone
The thyroid gland
Of the peripheral endocrine end-organs, as described above, the thyroid gland is one of the major endocrine glands in humans. The normal mature human thyroid exists of two lobes at both sides of the trachea, joined by the isthmus. Its name is derived from the Greek word for “shield gland” due to its topographic relationship to the laryngeal thyroid cartilage that resembles a Greek shield. The size of the thyroid, with a mass of about 10-20 grams in adults, depends on the quantity of dietary iodine.

In the thyroid, two specific cell types are present; the thyrocytes (follicular cells) and the C-cells (calcitonin-producing or parafollicular cells). The thyroid cells aggregate into follicles forming the basic structure of the thyroid, which is unique for endocrine organs. The follicles vary in size and contain proteins produced by the follicular cells. The shape of the follicular cells depends on the activated state of the cells, which is usually cubical to columnar. The cells are polarized with the apical membrane directed towards the follicular lumen. Iodide, taken up by the basolateral cell membrane, is transported through the cell and the apical cell membrane into the follicular lumen, where the organification of iodine and thyroid hormone production takes place. The lumen mainly contains the glycoprotein thyroglobulin (Tg), which has an essential role in the production of thyroid hormone and is an important reservoir for thyroid hormone and iodine.

The C-cells are situated either within the follicular wall immediately beneath the basal membrane or between follicular cells or they may form small groups of cells between the thyroid follicles. The C-cells derive from the neural crest; primordial cells migrate ventrally and become incorporated within the last (ultimobranchial) pharyngeal pouch. They move with the ultimo-branchial body to the point of fusion with the midline primordium that gives rise to the thyroid gland. The ultimobranchial body fuses with and is incorporated into the thyroid gland, and C-cells subsequently are distributed throughout the gland. The distinctive feature of C-cells, compared with thyroid follicular cells, is the production of calcitonin. Calcitonin is secreted continuously under
conditions of normocalcemia, but increases in response to an increase of the plasma calcium concentration.

Thyroid hormone
Thyroid hormone, T₄ and T₃, is essential for a large number of processes and functions in almost all tissues of the body. It is produced exclusively by the thyroid gland and is needed for growth and development and the metabolism of the cells ⁵. Antenatal and in the first few years of life it is essential for the development of the brain ⁶. The distinct features of a lack of thyroid hormone are best seen in people living in iodine-deficient regions, where severe iodine deficiency results in so-called endemic cretinism ⁷. The gross metabolic effects of a deficiency in thyroid hormone include reduction in oxygen consumption and changes in protein, carbohydrate, lipid, electrolyte, vitamin and hormone metabolism ⁸.

For the production of thyroid hormone, iodine is necessary. The amount of required iodine for an adequate thyroid function depends on body mass and the relative need: for neonates the recommended daily iodine intake is 50 µg/day and for adults this is 150 µg/day ⁹.

Iodide is actively transported from the blood into the thyrocyte by the sodium iodide symporter (NIS), an integral plasma membrane glycoprotein in the basolateral cell membrane of the thyrocyte. The transport of iodide by NIS depends on the presence of a sodium gradient across the basal membrane of the thyroid cell; the downhill transport of 2 Na⁺ ions results in the entry of one iodide ion against the electrochemical gradient. The synthesis of thyroid hormone can be divided into several steps (figure 1):

After uptake by NIS, I⁻ is translocated across the apical membrane towards the follicular lumen, mediated by pendrin and the apical I⁻ transporter ¹⁰. At the apical membrane outside the cell, I⁻ is oxidized by H₂O₂, catalyzed by thyperoxidase (TPO), in I⁻, bound as hypo-iodite in TPO. Hypo-iodite reacts with tyrosine residues in Tg resulting in mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) residues. TPO also catalyses the coupling of iodotyrosine residues in the Tg-molecule. Two DIT-residues form T₉, which is the main reaction ¹¹, and a DIT with a MIT form T₈. In this way, Tg contains MIT, DIT, T₄ and some T₃ residues in the peptide chains. After stimulation by TSH, Tg is endocytosed from the follicular lumen into the thyrocyte. The Tg-consisting
The biosynthesis of thyroid hormone (adapted from Physiol. Rev. 80: 1083-1105, 2000, with permission). Schematic representation of the biosynthetic pathway of thyroid hormones T₄ and T₃ in the thyroid follicular cell. Circles, active accumulation of I⁻, mediated by the Na⁺/I⁻ symporter (NIS); triangle, Na⁺-K⁺-ATPase; square, thyrotropin (TSH) receptor; diamond, adenylate cyclase; ellipse, G protein; cylinder, I efflux toward the follicular lumen; TPO, thyroid peroxidase (TPO)-catalyzed organification of I⁻; arrows, endocytosis of iodinated thyroglobulin (Tg), followed by phagolysosomal hydrolysis of endocytosed iodinated Tg and secretion of both thyroid hormones.

Endosomes fuse with lysosomes to phagolysosomes. In the phagolysosomes Tg is broken down to amino acids, including T₄, T₃, MIT and DIT. Also, T₄ is partially deiodinated to T₃ in the thyroid gland, due to the presence of deiodinase I and II in the thyroid. T₄ and T₃ are subsequently secreted into the circulation. The larger part of MIT and DIT is deiodinated and part of the I⁻ is re-utilized for Tg iodination.

After secretion into the blood, almost all of T₄ and most of T₃ bind reversibly to three transporting proteins: thyroxine-binding globulin (TBG), transthyretin (TTR) and albumin, of which normally the largest part binds to TBG. The function of the carrier proteins is most likely maintenance of a large extra thyroidal pool of thyroid hormone, of which only a small fraction of free hormone is immediately available to tissues. Normally, around 0.03% total plasma T₄, and 0.3% of the total serum T₃ are present in free or unbound form (free T₄ and free T₃).

NIS not only mediates active iodide transport into the thyroid gland, but its expression has also been detected in many other tissues such as the salivary glands, the gastric
mucosa, the placenta and in the lactating mammary gland, with the difference that these tissues can not organify accumulated I^- and that TSH exerts no regulatory influence on its accumulation. Iodide is further detected in ovaries and small amounts are detected in sweat and expiratory air. About 2/3 of the daily dietary iodine is excreted through the kidneys. Also 10-20 micrograms of I^- is lost via the faeces in the form of iodotyrosines.

Next to iodide, NIS transports several related monovalent anions, which are: TeO_4^-, ClO_3^-, ReO_4^-, SCN^-, BF_4^-, NO_3^-, Br^- and Cl^- Iodide uptake by NIS is inhibited by the different anions in order of affinity: perchlorate (ClO_4^-)> perthenate (ReO_4^-) > thiocyanate (SCN^-) > chlorate (ClO_3^-) > bromide (Br^-) As is seen, iodide is not the most highly selected anion. Even so, NIS can concentrate iodide from the circulation even if the concentration is very low (10^-8 to 10^-7 M). ClO_4^- is a 10-100 times more potent inhibitor than SCN^-, and its inhibition of NIS is also used in the perchlorate-discharge test to demonstrate iodide organification defects in the thyroid. Next to these anions and TSH, also certain cytokines (which may also inhibit Tg synthesis) and Tg suppress NIS activity (in vitro and in vivo).

1.1.c. The regulation of the thyroid: TSH, iodine and the Wolff-Chaikoff effect
The production of thyroid hormone is regulated by two major factors:
- the hypothalamic-pituitary-thyroid axis
- the circulating iodine content

The hypothalamic-pituitary-thyroid axis
As shortly described above, the production of thyroid hormone is regulated by the hypothalamic-pituitary-thyroid-axis, in which at least four steps can be distinguished:
1. The hypothalamus produces thyrotropin-releasing hormone (TRH)
2. TRH stimulates the adeno-pituitary to produce thyroid stimulating hormone (TSH)
3. TSH stimulates the thyroid to produce T_4 and T_3
4. T_4 and T_3 inhibit the secretion of both TRH and TSH.
Additionally, TSH secretion might be fine regulated by a short loop feedback at the pituitary level through the TSH-receptor in the plasma membrane of the thyrotropic cells. TSH has the most important external control on thyroid function. It acts through cyclic AMP and phospholipase C. TSH stimulates expression of the NIS gene, which increases the uptake of iodide into the cell. Furthermore, TSH stimulates iodide organification, thyroid hormone formation, Tg endocytosis, proteolysis and the release of thyroid hormones. Next to these metabolic effects, TSH stimulates thyroid cell proliferation (a.o. by modulation of the cAMP cascade) and increases the blood supply of the thyroid gland.

Iodine
To protect against the development of hyperthyroidism during exposure to an excess of iodine, by an overproduction of thyroid hormone, the thyroid gland has an intrinsic autoregulatory mechanism which responds to the intra-thyroidal concentration of iodine and is independent of TSH. In contrast, in case of iodine deficiency, the thyroid has several mechanisms to increase its efficiency of thyroid hormone production to prevent the development of hypothyroidism.
Excess iodine
The thyroid reacts to an excessive amount of iodine by the following ways:
- an acute inhibition of the vascularization (this phenomenon called “plummering of the thyroid” is also used to reduce the size of the thyroid preceding surgery17).
- an acute extra release of T₄ and T₃
- a decrease in the response to TSH³
- an inhibition of H₂O₂ production and inhibition of TPO resulting in a block of iodide organification18,19. This phenomenon is also known as the Wolff-Chaikoff effect, named after two of the scientists by whom it was first reported in 194820. This phenomenon is thought to go together with the formation of an organic iodocompound (XI, iodolacton or alpha-iodohexadecanal)21. The exact metabolic basis for the Wolff-Chaikoff effect has still not yet been elucidated19. It has been demonstrated that the acute inhibition of the iodide organification due to iodine excess can be suppressed by the administration of anti-thyroid drugs, indicating that the formation of compounds that are responsible for this effect (XI or iodolacton) both require prior oxidation of iodide to a reactive form22.

Figure 3. Effects of iodide on thyroid metabolism. (from The Thyroid and its Diseases, chapter 1, www.thyroidmanager.org, with permission). The various steps inhibited by I⁻ (indicated by /). Three possible mechanisms are outlined, the generation of O₂ radicals, the synthesis of a XI compound, and the iodination of target proteins.

Iodine excess has several more effects on the thyroid gland, either directly or indirectly through compound XI, as shown in figure 3. This comprises inhibition of TSH signal transduction via cAMP or DAG pathways, the glucose transport and oxidation, lactate production, calcium efflux, and blocks endocytosis22.
One of the steps that is inhibited by XI is uptake of iodide into the cell. By this mechanism, the thyroid can escape or adapt to the Wolff-Chaikoff effect. The decreased influx of iodide is caused by a decrease of NIS mRNA. After lowering of the intrathyroidal concentration of iodide, subsequently also the production of XI or iodolacton is ceased, and the thyroid hormone production will be restarted \(^{23,24}\). This autoregulation, which lasts for 26 to 50 hours, has been named the escape-mechanism. In this way, through the combination of the Wolff-Chaikoff effect (block in iodide organification) and its escape mechanism (decrease of NIS activity), euthyroidism is restored while exposed to prolonged iodine excess.

Despite this auto regulatory system, iodine excess may result in hypothyroidism or hyperthyroidism. In individuals that lack the escape-mechanism or that have an inefficient escape-mechanism, iodine excess will lead to the development of hypothyroidism due to a continuous blockade of the organification. This has been described in individuals with hypothyroidism and seaweed goiters \(^{22}\). Also, iodine induced hypothyroidism can often be observed in fetuses and (preterm) neonates, because the escape mechanism of the Wolff-Chaikoff effect develops in the last 4 weeks of pregnancy \(^9\). In the elderly, hypothyroidism and goiter are frequent during exposure to excess iodine, probably due to a higher incidence of auto-immune thyroiditis and/or defective organification. Furthermore, iodine excess causes hypothyroidism in a part of the patients with underlying thyroid diseases, such as Hashimoto’s thyroiditis, post-partum thyroiditis, Graves’ hyperthyroidism or after partial thyroidectomy \(^9\).

Iodine induced hyperthyroidism (Jod-Basedow disease) was first described after the administration of dried seaweed in an iodine deficient region in France. This is most probably caused by an excessive production and release of thyroid hormone by pre-existing autonomous nodules. In iodine sufficient regions, iodine induced hyperthyroidism is very rare, but it may be seen in elderly and patients with non-toxic goiter \(^9\).

Also medication containing iodine can cause hypo- or hyperthyroidism partially by the same mechanisms, and are described in section 1.3.b and in table 1.

**Iodine deficiency**

In case of iodine deficiency, the thyroid increases the efficiency of thyroid hormone production, mostly mediated by increased TSH levels, in the following ways:
the iodide uptake is increased by activation of NIS; in iodine sufficient or repleted
countries the iodide uptake may be 10-30% in 24-48 hours, in iodine deficient
countries this can be increased up to 80% in a few hours.
- the thyroid shifts its production towards T₃ at the expense of T₄, due to increased
thyroidal expression of both D1 and D2, and thus increased intrathyroidal T₄ to
T₃ conversion. Also the amount of available DIT may be lower.
- the iodine turnover is more rapid, little Tg is stored in the follicular lumen and thus
iodine is not “wasted” in storage.
- to conserve iodine, it is recycled within the thyroid gland.
- its vascularization is increased, independent of plasma TSH.
- the thyroid mass is increased, which is presumably effective for producing more
thyroid hormone a.o. by competition with the renal clearance. Continuous
stimulation by TSH, however, may, in time, lead to a more heterogeneous thyroid
with nodules and less productive action.

1.1.d. Iodothyronine deiodinases
In normal thyroid glands, mainly T₄ and a small amount of T₃ are produced. T₄ functions
primarily as prohormone and is converted to T₃ by iodothyronine deiodinase. T₃ has the
most metabolic effects, due to its strong affinity for the nuclear receptor. The conversion
to T₃ occurs by enzymatic outer ring deiodination of T₄. Inner ring deiodination of T₄
results in the metabolite reverse T₃ (rT₃). Normally about one-third of T₄ is converted

![Figure 4. Deiodination of T₄ through T₃ to T₂](image)
to T₃ and about one-third to rTᵧ. T₃ and rT₃ are both further metabolized, mainly to the metabolite 3,3'-T₂. The 3 enzymes catalyzing these deiodinations are type I (D1), type II (D2) and type III (D3) iodothyronine deiodinases. Expression of D1 and D3 is under positive control and that of D2 is under negative control by thyroid hormones. This implies that the contribution of D1 and D2 to the peripheral T₃ production is dependent on the thyroid state, with D1 prevailing in the hyperthyroid and D2 in the hypothyroid state.

The clinical importance of the deiodinases in the regulation of thyroid hormone bioactivity is noticed for example during conditions such as non-thyroidal illness and malnutrition. In non-thyroidal illness (NTI) a decreased concentration of plasma T₃ is found with an increase in plasma rTᵧ. Plasma FT₄ remains usually within normal limits. The changes in plasma T₃ and rTᵧ can be explained by a diminished conversion of T₄ to T₃ and of rTᵧ to 3,3'-T₂ by D1 in the liver, which has recently been explained by a decreased D1 expression in the liver. A diminished activity of transporters mediating uptake of T₄ and rTᵧ in the liver appears to be another important mechanism. In patients with large hemangioma's, subclinical or even overt hypothyroidism may be present, of which it has been demonstrated to be induced by the expression of high D3 activity ("consumptive hypothyroidism"). Furthermore, several drugs can inhibit the peripheral production of T₃. Examples are dexamethasone, propranolol, and iodinated compounds such as the anti-arrhythmic drug amiodarone.

1.1.e. Pediatric thyroid disorders
Thyroid disorders during childhood can be divided into congenital and acquired diseases.

Congenital thyroid diseases
Congenital hypothyroidism (CH) can be the consequence of iodine deficiency, thyroid dysgenesis (around 70% of cases in iodine sufficient areas), inborn errors of thyroid hormonogenesis (15% of cases) or due to pituitary or hypothalamic disorders (15%). It occurs frequently in children with Down syndrome. Recently, a cohort of newborns with central CH has been described who appeared to be born from mothers with undetected Graves’ disease.
Because thyroid hormone can be transferred over the placenta until the term date, the newborn with CH is often phenotypically normal. Because low thyroid hormone concentrations early in infancy can result in mental and motor retardation and the recognition of CH is clinically difficult, a neonatal screening has been introduced. In the Netherlands, screening is based on the determination of plasma $T_4$ with additional determination of TSH in the 20% samples with the lowest $T_4$ concentrations ($T_4 \leq -0.8$ SD). In the $T_4$ values of $\leq -1.6$ SD also the $T_4/TBG$ ratio is measured to exclude low $T_4$ due to TBG deficiency. The occurrence of CH is about 1:3100 newborns. Congenital hyperthyroidism is mostly the result from transplacental passage of maternal TSH receptor stimulating antibodies and is almost always transient. Permanent congenital hyperthyroidism is very rare. One case has been described in which a germline mutation resulted in TSH receptor activation.

Acquired thyroid diseases
Acquired thyroid diseases can be divided into hypothyroidism, hyperthyroidism, nodules, goiter and thyroid carcinoma.

Acquired hypothyroidism can be of thyroidal or central (pituitary or hypothalamic dysfunction) origin. Causes for acquired thyroidal hypothyroidism can be autoimmunity, iodine deficiency, drugs, radiation and surgery. Auto-immune thyroid disorders can be the result from Morbus Hashimoto, it can be found in relation with Diabetes Mellitus (DM), as part of the autoimmune polyglandular syndrome or it can be associated with chromosomal abnormalities such as Down, Turner or Klinefelter syndrome. The effects of drugs, radiation and surgery on the thyroid are discussed later in this introduction (sections 1.3, 1.4 and 1.5).

Acquired damage to the pituitary or hypothalamus can be the result of tumors (particularly craniopharyngioma, glioma and Langerhans' cell histiocytosis (LCH)), granulomatous disease, cranial irradiation, infection (meningitis), surgery or trauma. Usually the synthesis of other tropic hormones are also affected, particularly growth hormone.

The consequences and clinical signs of hypothyroidism include mental and motor retardation in the very young, lethargy, cold intolerance, constipation, dry skin and/or hair texture, periorbital edema, growth retardation, goiter and delayed or even
precocious puberty. Other metabolic consequences are secondary dyslipidemia (a.o. increased total cholesterol, VLDI. and LDL due to a reduced number of LDL receptors in the liver and decreased biliary excretion of cholesterol) and an elevated plasma phosphate concentration 41.

Laboratory evaluation is done by measuring TSH in combination with free T₄ (FT₄). Thyroidal hypothyroidism is diagnosed when FT₄ levels are low in combination with an increased level of TSH. In case of central hypothyroidism, the concentration of TSH remains low to normal despite low circulating levels of FT₄. Additional diagnostic information can be obtained by measuring anti-peroxidase antibodies (anti-TPO), T₃, T₉, rT₉, TBG, Tg and by performing a TRH test (TRH 10 µg/kg, with a maximum (adult) dose of 200 µg). In normal individuals the peak TSH secretion is found after 15-30 minutes, with a return to normal values after 3 hours. In hypothalamic hypothyroidism a delayed peak is found after 60-90 minutes, without a return to the baseline value. In pituitary hypopituitarism, there is (almost) no TSH response 31.

Treatment for hypothyroidism consists of the supplementation of T₄, with adequate monitoring of plasma TSH and FT₄, growth and pubertal development. The combination of a TSH elevation (TE) with serum FT₄ and T₄ levels within the normal range is called compensated or subclinical hypothyroidism. Subclinical hypothyroidism is relatively common in adults (approximately 6 % in the general population, more in females than in males 41), but the clinical consequence of this finding is still an issue of debate. Because the T₄ set-point is different between persons, a plasma FT₄ level within the normal range may be too low for the individual patient causing TSH to rise. The risk of progression into overt hypothyroidism enhances with increasing TSH levels and with circulating auto-antibodies 41,42, and approximately 2-15% per year will progress into overt hypothyroidism 41,43. The occurrence of secondary dyslipidemia, cardiac dysfunction and arteriosclerosis has been suggested but up to now it has not been demonstrated in patients with subclinical hypothyroidism 40,43. In children, subclinical hypothyroidism can be associated with auto-immunity (for instance in Down syndrome 44 or DM 45), is seen after exposure to radiation 46,47 and iodine deficiency 48. In children with DM, an association with symptomatic hypoglycemia has been made 45.
In general, the strength of evidence for treatment of subclinical hypothyroidism in children is not strong. Treatment, to prevent progression into overt hypothyroidism and dyslipidemia, is advised when TSH values are > 10 mU/L, or when circulating auto-antibodies or dyslipidemia are present. In pre-pubertal children with DM, T4 supplementation for subclinical hypothyroidism resulted in significant improvement in growth velocity.

After exposure to radiation, it has been suggested that a prolonged TSH elevation is not preferable and, theoretically, the risk to develop thyroid carcinoma is diminished by keeping the stimulation by TSH weak. For this reason, it is suggested to keep the level of TSH in these children within normal values (0.4-4 mU/L), however there is no long term evidence to support this (discussed in chapter 11 and 12).

Acquired hyperthyroidism is most frequently caused by Graves' disease, an auto-immune disorder. Other rare causes are autonomous functioning adenoma, a constitutive activation of the TSH receptor or as part of the McCune-Albright syndrome. Central hyperthyroidism can be the result of a TSH-secreting pituitary adenoma or a selective pituitary resistance to thyroid hormone. Treatment can be medical, surgical or with radiation (external or radio-iodide).

Goiter is caused by the generation of new thyrocytes and follicles, caused by stimulation of TSH, growth-stimulating immunoglobulins, Insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF). Worldwide, the main cause in children is iodine deficiency. Furthermore, it can be caused by auto-immunity or it can be a 'simple' non-toxic or colloid goiter. Goiter can be associated with euthyroidism, hypothyroidism and hyperthyroidism. Some colloid goiters regress spontaneously, others undergo periods of growth and regression, resulting ultimately in the large nodular thyroid glands (multinodular goiter) later in life.

Thyroid nodules (a lump in the thyroid, or “an area with a different texture than the normal parenchyma”) are not frequently seen in the first 2 decades of life, but when they are found, in children they are more likely to be carcinomatous than in adults. The occurrence of nodules may be present in 2.0-5.6 % (the latter in borderline iodine sufficient areas) in healthy children. In a survey performed in 1995 and 1996 in the Netherlands, the reported incidence of nodules in 937 healthy schoolchildren was
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found to be 1.2%. This study was performed in 1995-1996, and it must be remarked that in the past 10 years the sensitivity of ultrasonography has improved and perhaps, now, in 2004, nodules can be detected more sensitively than 10 years ago.

Impalpable thyroid nodules that are found by ultrasound imaging by chance, have been called thyroid incidentaloma. The clinical significance of thyroid nodules and incidentaloma is debatable; in a cohort of 267 adult patients (mean age 51 years, range 26-75), 317 incidentally found nodules were aspirated of which 44 (13.9%) proved to be malignant. None of these patients had been irradiated. This demonstrates that malignancies may be present, but, considering the fact that the prognosis of occult thyroid cancer is quite good, the impact remains debatable. In 8.6% of autopsy cases, latent carcinoma of the thyroid was found. For children, as their lifespan is longer, the clinical impact of thyroid incidentaloma may be different. Factors which can be a risk factor to develop thyroid nodules include iodine deficiency, irradiation and infection. The size, consistency and possible existence of hypo- or hyperthyroidism should be investigated.

Imaging with $^{99m}$Technetium pertechnetate ($^{99m}$TeO$_4^-$), $^{123}$I$^-$ or $^{131}$I$^-$ can be done to judge whether the nodules are hot (functioning) or cold (hypo/non-functioning). In presence of an elevated TSH (which can be elevated due to the presence of thyroidal hypothyroidism or which can be given iv as recombinant) imaging is most clear.

The choice of the imaging agent depends on several factors. $^{99m}$TeO$_4^-$ (t$_{1/2}$ of 6 hours) is taken up but not organified and quickly disappears from the thyroid gland. It is cheap and delivers only a small radiation dose to the thyroid gland. $^{131}$I has a longer t$_{1/2}$ (8.1 days), is mainly a β- and also a γ-emitter. $^{123}$I has a short t$_{1/2}$ (0.55 days), is expensive, but is very well detected by γ-cameras and delivers about 1% of the radiation dose that is delivered by $^{131}$I. For these reasons, $^{123}$I is the isotope of choice for uptake studies in children. Benign nodules can be functioning or hypo-functioning, but malignant nodules are almost never “hot”.

Fine needle aspiration cytology (FNAC) is the most useful and diagnostic procedure to obtain information on the nodular features. The presence of malignant cells is independent of the size of the nodule. It has been shown that T$_4$ or iodide supplementation can diminish the size of the nodule, most effectively in young
patients. However, it is still debatable whether it is indicated to treat nodules. Thyroid nodules after irradiation are discussed in section 1.4 and chapters 3, 11 and 12. Thyroid carcinoma are discussed in section 1.2 and chapters 7 and 8.

1.2: Pediatric malignancies of special importance for this thesis

General

Compared to cancer in adults, cancer in children is rare. In the Netherlands, in total around 400 children to the age of 16 years are diagnosed with a malignancy every year. In the years 1989-1997, in the Netherlands, the groups of malignant diseases that were diagnosed in children were leukemia (27.6%), brain tumors (19.1%), lymphoma (11.3%), Wilms' tumor (6.7%), neuroblastoma (5.1%), rhabdomyosarcoma (4.6%), other sarcoma's (3.9%), retinoblastoma (3.6%) osteosarcoma (2.4%) gonadal carcinoma (2.2%), Ewing sarcoma (1.6%), thyroid carcinoma (1.2%) and skin melanoma (1%).

Tumor genesis in adults and children is different. In many neoplasms in adulthood, a multistep process has been shown to take place during tumor development and progression, of which the development of adenomas of the colon into adenocarcinomas is one of the best examples. Because pediatric cancers arise early, its origin is dominated by genetic inheritance and familial predisposition, more than environmental factors. In sporadic pediatric tumors, only few or even single initiation events, such as specific translocations and subsequent oncogene activation or deletions leading to loss of tumor suppressor function are responsible for tumor development. A special category is the secondary malignancy in a childhood cancer survivor, which is often induced by ionizing radiation (see section 1.4).

The prognosis for a child diagnosed with a malignant disease depends mainly on the tumor-type and stage of disease. The overall 10-year survival for children with leukemia is in the range of 80%, but for neuroblastoma stage IV it is only 20-30%. In general, the overall 10-years survival for children with cancer in the Netherlands has improved over the years to an overall percentage of 70%, thanks to new treatment strategies and developments in scientific research.
Due to the fact that the numbers of long term survivors increase, the late effects of the disease in relation to the given treatment are more profound and need serious interest. This initiates the discussion of the ethical boundaries of treatment in relation to quality of life. Examples are adjustments of the standard prophylactic cranial irradiation which is given to prevent central relapse of ALL in young children because of its serious effects on the mental development and the awareness of clinical heart failure due to treatment with anthracyclines.

In this thesis, special attention will be paid to the endocrine effects of treatment for neuroblastoma, differentiated thyroid carcinoma and medullary thyroid carcinoma. For this reason, these tumors will be described in more detail.

1.2.a. Neuroblastoma

Neuroblastoma (NB) is an embryonic tumor of the sympathetic nervous system, derived from the primitive neural crest. Of all childhood solid tumors, NB is known with the broadest spectrum of clinical behavior. Some tumors (stage IVs) regress spontaneously, some can be cured using chemotherapy and others are resistant even to very intensive chemotherapy.

NB accounts for 5-10% of all childhood cancers. Per 100,000 children under the age of 15 years, there are 1.3 new cases per year. The peak age of incidence is between 0 and 4 years, with a median age of 23 months. Forty percent of children are under one year of age at diagnosis.

The origin of NB is thought to be in the immature neuroblast cells, which are primitive neural crest cells. The neural plate is formed in the ectodermal germ layer, in the 3rd week of gestation. After fusion of the neural ridges into the neural tube, the neural crest is formed. The neural crest cells form the sympathetic peripheral neural system, the facial skeleton, the thymus, the parathyroids, the enteric nervous system and the skin melanocytes.

The mature sympathetic nervous system consists of a neuronal part and a hormonal part, that both produce catecholamines, as neurotransmitter or as hormone.

In the development of the neural crest, segmentation and migration are characteristic...
phenomena. The primitive neural crest cells (neuroblasts) migrate to a position lateral to the neural tube, forming primitive ganglia on both sides of it. Eventually they form the para-ganglia (ventral to the spine), visceral sympathetic ganglia (abdominal organs) and the adrenal medulla. In all these localizations, NB can develop (figure 5). The more differentiated sympathetic cells (ganglion cells) can not only give rise to the development of NB, but also to ganglioneuroma. The differentiated hormone-producing cells (chromaffin cells) can also give rise to the development of pheochromocytoma.

Histologically, two cell types can be distinguished in NB; the neuroblast/ganglion cell and the Schwann cell. For undifferentiated NB, the presence of a small round blue cell tumor is very typical. In the International Neuroblastoma Pathology Classification (INPC), 3 types NB are distinguished: NB (undifferentiated, poorly differentiated or differentiating), ganglioneuroblastoma (intermixed or nodular) and ganglioneuroma. The clinical symptoms of NB can be caused by the primary tumor, the metastases, or can be a paraneoplastic phenomenon. The primary tumor can be localized at any site of the normal sympathetic nervous system structures, e.g. adrenals, sympathetic chain.
or the abdominal paraganglia, as shown in figure 5. About 25% of the NB are found in the neck and thorax, 70% in the abdomen and 5% in the pelvis. Metastases occur mostly in bone (presenting with a limp), bone marrow (presenting with anemia or thrombocytopenia), lymph nodes (presenting with lymphadenopathy), or in the orbit (presenting with proptosis and peri-orbital ecchymoses). Paraneoplastic signs can be an opsomyoclonus or a secretory diarrhea mediated by vasoactive intestinal polypeptide (VIP).

The diagnostic criteria have been defined by the International Neuroblastoma Staging System (INSS) working party. Stages 1 and 2 are localized disease, stage 3 is unresectable NB and stage 4 presents with any primary tumor with dissemination of disease. A special stage is 4s, which is only diagnosed in children before age 1. This stage also shows a localized primary tumor with dissemination limited to the skin, liver and/or bone marrow, but has an excellent prognosis and may even regress spontaneously.

The diagnosis is made by the finding of a solid tumor at physical examination suspect of NB, and can be confirmed by raised excretion of catecholamines in the urine, elevated serum LDH and ferritin, bone marrow evaluation, a positive radio-MIBG scan and a tissue biopsy. Additional investigations can be done by CT, MRI, ultrasound and 123I-Meta-iodobenzylguanidine (MIBG)-administration.

The treatment is based on age, stage and N-Myc copy numbers. Localized tumors, at an age of less than 2 years and no N-myc amplification do not need more than surgical resection or sometimes even less than that. All the other patients need intensive multimodality therapy.

Different treatment protocols are currently being followed. One of the main differences in treatment protocols for stage 3 unresectable and stage 4 tumors in children older than 1 year is the possibility of treatment with 131I-MIBG, which is given in several centers in the world. The goal of 131I-MIBG treatment before surgery is to replace the pre-operative chemotherapeutic regimen, so that less toxicity is obtained before surgery and more room is left for cytotoxic treatment after surgery. This treatment has been given since 1989 in Emma Children's Hospital, AMC, as “upfront” therapy, followed by chemotherapy, surgery and ABMT (further discussed in section...
1.4 and chapters 2, 3 and 4). Also, as adjuvant palliative therapy for recurrent NB, \(^{131}\)I-MIBG is given, resulting in temporary remissions and prolongation of life.

The prognosis for children with NB depends amongst others on the stage of disease, age, the presence of N-myc amplification and the loss of tumor-suppressor genes (chromosome 1p). The N-myc oncogene is present in 25-35\% of NB, which results in a strong overexpression of N-myc mRNA and protein, causing a highly aggressive behavior and a bad prognosis.\(^2\) Chromosome 1p loss is more frequently observed in stage 3 and 4, and is correlated with increased plasma ferritin and LDH concentration. In general, the prognosis for stage 1 and 2 survival is very good (90 and 85 \% respectively).\(^6\)

When N-myc amplification is present, however, only 50 \% will survive. In stage 3, long-term survival is reported around 65 \%. For stage 4 patients, the prognosis is one of the worst of all pediatric malignancies, with a 5-year survival rate of 25 \%.

1.2.b. Thyroid carcinoma

Thyroid carcinoma in children is very rare, with an annual incidence of 0.02-3.0 per 100,000.\(^4\) In the Netherlands in the years 1989-1997 103 children < 19 years were diagnosed with thyroid cancer.\(^6\) Thyroid carcinoma is more frequent in girls than in boys (3.2:1).\(^4\) Histologically, four types of thyroid carcinoma can be distinguished: follicular, papillary, medullary and anaplastic.

Differentiated thyroid carcinoma

Differentiated thyroid cancer (DTC) is mostly sporadic, although some familiar cases have been described. Also, several syndromes are known with an increased incidence of thyroid cancer, for example Gardner's syndrome (familial adenomatous polyposis coli) and Cowden disease.\(^5\)

In children, of the differentiated carcinoma, the papillary carcinoma (PTC) or the follicular variant of papillary carcinoma is the most dominant histologic type (69-94 \%).\(^5\) This type of adenocarcinoma typically shows tumor cells around a fibrovascular core and, not infrequently, areas of follicular differentiation. The papillary lesions are often infiltrative, and encapsulation is rare. Lymphocytic "reactions" and psammoma bodies, spiral rings of calcification, are prominent. The cell nuclei have a ground-glass or
“cat’s eye” appearance and intracellular inclusions are common. Vascular invasion is rare. Many tumors look much like follicular cancers, but have the characteristic nuclei of papillary cancers. These constitute the “follicular variant” of papillary cancer. Over the years, PTC has been associated with continuous elevated levels of TSH, previous Hashimoto disease, exposure to ionizing irradiation and mutations in the RET proto-oncogene.

In children, exposure to irradiation is the only proven causal factor to promote the development of differentiated thyroid carcinoma. Radiation-induced thyroid carcinomas are discussed in section 1.4.

Follicular carcinoma (FTC) in children has been reported in the range of 0-30% of all diagnosed thyroid tumors in the pediatric age group. Follicular adenocarcinomas vary from those with a definite follicular pattern to those with solid sheets of cells. The lesions are more frequently encapsulated, but capsular and blood vessel invasions are typical, which can lead to spreading to lungs and bones. The nuclei are normo- or hyperchromated, or may be quite vesicular.

The classification of thyroid cancer, despite the use of standardized nomenclatures and standard histological criteria, is difficult and inter-observer variation has been reported to be 7% for PTC and 27% for FTC. The anaplastic carcinoma is very rare in children and is associated with a bad prognosis. As these carcinomas will not be dealt with in this thesis, this type will not be discussed.

Molecular genetic studies have shown aberrant expressions of the RET proto-oncogene to be present in PTC. In the sporadic tumors that are RET/PTC negative, a high prevalence of BRAF mutations was found recently. Also, an increased expression of active phosphorylated MAP kinase has been demonstrated in PTC, modulating cellular proliferation. NTRK1 is another gene often activated in PTC. In adults, activation of RAS and GSP is frequent, mainly in FTC, but also in PTC. In children, however, only a sporadic mutation of RAS has been described (after radiation-exposure) in FTC, and these oncogenes do not appear to play a role in the pathogenesis of sporadic pediatric thyroid carcinoma. Mutations of p53 are most frequently found in anaplastic carcinoma, but rarely in PTC and FTC, and are probably important in the dedifferentiation process.
The clinical features and outcome in pediatric populations are comparable for FTC and PTC types, although they are distinct pathological entities. The presentation is in 60-80% a palpable mass in the thyroid region or lymphadenopathy, and the disease is often in a more advanced stage when compared to adults. Lymph node metastases are found in approximately 74% and lung metastases in 18%. Bone and central nervous manifestations are very rare.

Due to the fact that differentiated thyroid carcinoma in children is an uncommon malignancy, the treatment strategy is still controversial regarding the optimal initial and subsequent long-term treatment and follow-up. For the majority of patients, total thyroidectomy with or without lymph node dissection is recommended. However, in case of a unilateral tumor with no metastases, in some centers a hemithyroidectomy is performed. Chemotherapy plays no role in the treatment of these tumors. Adjuvant therapy, is given with radio-iodide to ablate residual normal thyroid tissue after subtotal thyroidectomy or lobectomy and to treat functioning metastases. After ablation therapy, plasma Tg levels can be used as tumor marker for monitoring tumor progression of recurrence. For follow-up of low-risk adult patients (no distant metastases, stage T4-tumors, poorly differentiated histotypes or incomplete surgery), with no evidence of disease up to a 12-month period, a recent protocol advises the use of plasma Tg after recombinant TSH administration during follow-up and ultrasonography to detect neck recurrences, without additional diagnostic whole body scanning. For follow-up of high-risk patients, special protocols should be followed.

The prognosis of differentiated thyroid carcinoma is very favorable with extremely low mortality rates. The chance for survival after thyroid carcinoma for children and adolescents is better than for adults, despite its more advanced presentation. Recurrence, however, is seen frequently and can become manifest decades after the first diagnosis. Late effects of the tumor and treatment are discussed in chapter 7.

Medullary thyroid carcinoma

Medullary thyroid carcinoma (MTC) is a different type than differentiated carcinoma, which derives from the parafollicular, or C-cells, of the thyroid gland. It occurs sporadically in about 70-80% of cases and in 20-30% it is a familiar tumor (as part of the endocrine syndromes Multiple Endocrine Neoplasias (MEN) type 2A and 2B or
as familial MTC not associated with MEN. The familial forms are inherited as an autosomal dominant trait. Distinct germ-line mutations in the RET proto-oncogene, mapped in the pericentromeric region of chromosome 10, have been identified in patients who are affected with familial MTC. MTC is the first neoplastic manifestation in most MEN2 kindreds due to its early and overall higher penetrance. MEN type 2B is the most aggressive and distinctive of the MEN2 variants, with MTC in 100% and pheochromocytoma in 50%, but no parathyroid disease. Also, these patients have a decreased upper/lower body ratio, a marfanoid habitus, mucosal and intestinal ganglio-neuromatosis. In MEN 2A, MTC (90 to 100%), pheochromocytoma (50%) and parathyroid tumors (20-30%) are found. Metastases of MTC may be in the central and lateral, cervical and mediastinal lymph nodes or more distantly in lung, liver, or bone.

As MTC is not sensitive to chemotherapy or to radiotherapy, total removal by surgical resection is the only curative option. Calcitonin is the most specific circulating and immunohistochemical marker for MTC. If basal values or pentagastrin stimulated values are elevated, surgery must be performed as soon as possible.

In case of a known mutation of the MEN syndrome, the thyroid is removed prophylactically. Due to the risk associated with delaying thyroidectomy, the morbidity of early surgery is outweighed. In MEN 2B patients, thyroidectomy should be performed before the age of 6 months to 1 year, in case of MEN2A prophylactic surgery is recommended before the age of 5 years. However, MTC has also been found in MEN2A patients of only 1 year of age, indicating that MTC in MEN2A can also be very aggressive. The policy regarding central lymph node dissection at initial thyroidectomy is controversial. After surgery, elevated or increasing values of CT are generally the first sign of persistent or recurrent disease. In carriers of mutations in RET, codon 634, nodal metastases have been demonstrated to occur after an average time of 6.6 years after malignant transformation. For this reason, it has been advised to follow the levels of CT for detection of recurrent MTC, basal and after calcium or calcium/pentagastrin stimulation, after 2 and 5 years and at longer intervals if stimulated calcitonin levels are unchanged. Several studies have reported on the long-term outcome of children after prophylactic thyroid surgery for MTC. It was seen that patients given a
thyroidectomy following genetic testing versus following diagnosis of MTC had a lower tumor load. In 18 children followed for a mean period of 3 years after prophylactic thyroidectomy, all stimulated calcitonin levels were nearly undetectable. Of 207 patients with a RET point mutation, 4 had elevated calcitonin levels on pentagastrin simulation after thyroidectomy, of which in 3 patients nodal metastases were found.

In case of elevated calcitonin levels, subsequent localizing of the tumor can be done using CT-scanning, ultrasound, MRI and bone scintigraphy. More specific nuclear compounds have been developed to trace MTC, such as $^{123}$I-MIBG, $^{201}$Thallium-chloride, $^{99}$Tc-pentavalent-dimercapto-succinic acid, radio-labeled somatostatin analogues, monoclonal antibodies directed against calcitonin (anti-CT) or carcino-embryonic antigen (anti-CEA), $^{18}$F-fluoro-D-deoxyglucose-positron emission tomography (PET) and somatostatin receptor scintigraphy with $^{90}$mTc-FEDDA/HYNIC-TOC. However, despite all these techniques, localizing the metastases can be extremely difficult. External radiotherapy and chemotherapy can be given as palliation. Radiation therapy with radio-iodide is not indicated, as the C-cells do not trap iodide. Nuclear medicine approaches that may be used for therapy (if the cells take up the compound) are $^{131}$I-MIBG, $^{111}$In-pentetreotide and monoclonal antibodies against CEA coupled to $^{131}$I. The somatostatin analogs (octreotide, lanreotide and interferon-α) have been administered therapeutically in advanced stages of MTC, and appears to have a symptom-relief effect and show low toxicity with a very good compliance. Selective venous catheterization to localize the tumor after pentagastrin stimulation by measuring CT268 by multiple venous sampling can be done, with extensive surgical exploration of the area in which levels are found. As the gain-of-function mutation in the RET-proto-oncogene leads to an increased activity of tyrosine kinase and cell growth, the use of protein-tyrosine kinase inhibitors has been studied in vitro demonstrating inhibition of cell growth and RET tyrosine kinase activity. Trials using genetic cytokine immunotherapy give promising results, but dose-dependency and systemic toxicity are a problem in studies done both in animals and in man. For this reason, effective gene therapy using recombinant adenovirus has been studied. In animal and in vitro studies, it has shown to be effective with less toxicity. In humans, this has not been tested yet.
In chapter 8, a case-report is presented of the difficulties that can be met, when no tumor can be detected while the circulating levels of calcitonin are extremely high after early thyroid surgery in MEN-2A syndrome.

1.2.c. Endocrine effects caused by a pediatric malignancy
The endocrine system may be distorted by a pediatric malignancy, dependent on the localization of the tumor. For example, the function of the hypothalamus and/or pituitary gland may be damaged in presence of a brain tumor, like medulloblastoma, craniopharyngioma, prolactinoma, hamartoma or astrocytoma, resulting in one or more deficiencies or overproduction of the hypothalamic and/or pituitary hormones. In 43% of brain tumor patients, TSH abnormalities were found before starting treatment (irradiation). Tumors can also be located in one of the peripheral endocrine organs, such as the thyroid, testis, ovary, or the adrenal gland causing dysfunction of these organs resulting in either hyper- or hypoactivity. However, the function can also be completely normal in presence of a tumor. Furthermore, an endocrine disorder may be the presenting sign of a pediatric malignancy, as paraneoplastic sign, due to the production of hormone-agonists. An example is the production of HCG causing precocious puberty in children with a germ cell or choriocarcinoma.

1.3: Chemotherapy and endocrine effects
1.3.a. General
Anticancer drugs are of great importance for pediatric oncology and contribute to a marked increase in the cure-rate. In chemotherapy, single drug procedures are used, but most often drugs are given in combinations (multi-drug regimen). The mechanisms of action of most anti-neoplastic drugs consists of interference with the synthesis or function of DNA.

Cytotoxic drugs have a narrow therapeutic range and are only effective when high, toxic concentrations are reached. The dose of the drug that should be administered is calculated from the body weight or body surface of the patient. The clearance of a drug is determined by many factors, and shows a high interindividual variability. This is one of the reasons why the same dose administered to different children causes a
relatively low toxicity in some, but show more serious toxic effects in others\textsuperscript{113}. All the cytotoxic drugs that are used (in pediatric oncology) can be divided into subgroups, based on their mode of action. These subgroups will be discussed shortly, together with the main toxicities caused by these drugs\textsuperscript{113,114}. The generic names of the drugs in each subgroup are shown in table 1 of chapter 10.

- \textit{Alkylating agents:}
These agents are derived from mustine and alkylsulfonic acid. Their most important action is irreparable damage to DNA, largely independent of the cell-cycle, by binding to proteins and nucleic acids, by which covalent cross-links are formed between two complementary DNA-chains. This causes an interruption in the DNA replication. Its most important acute toxicities are myelosuppression and hemorrhagic cystitis (cyclophosphamide/ifosfamide).

- \textit{Anti-neoplastic antibiotics or anthracyclines:}
These agents are produced by micro-organisms and interrupt the synthesis of DNA, RNA and proteins by binding to DNA. Furthermore they inhibit topo-isomerase II, form free radicals and may have effects on the cell membrane structure. The anthracyclines are the most frequently used anticancer drugs in children and in adults. Toxicity can be myelosuppression and cardiac failure. Bleomycine, one of the anti-neoplastic antibiotics, can give serious lung toxicity.

- \textit{Anti-metabolites:}
These agents interrupt the biosynthesis or the function of nucleic acids, due to changes in molecular structure. The purine and pyrimidine-antagonists are competitive with the regular nucleic acids due to their structural resemblance. The folic acid-antagonist inhibits the reduction of dihydro-folic acid and inhibits the synthesis of the basic compounds of nucleic acids. Reported toxicities are myelosuppression, hepatotoxicity and nephrotoxicity.

- \textit{Plant alkaloids (vinca-alkaloids):}
The vinca-alkaloids, gained from Vinca Rosea, bind to intracellular tubuline-proteins and inhibit the formation of microtubuli. These are essential for cell motility,
intracellular transport and mitosis. The taxanes stimulate the synthesis of microtubuli from tubulinedimers and stabilize the microtubuli, through which the cell is specifically inhibited in its division. The main dose restriction is caused by neurotoxicity (vincristine) and myelosuppression (vinblastine).

- **Platinating compounds:**
The platina-containing compounds inhibit DNA-synthesis by the formation of platina-crosslinks between DNA-chains (platina-DNA-adducts). The most important side effects are nephro-, neuro- and ototoxicity (mainly caused by cisplatin) and hematotoxicity (trombopenia, mainly after carboplatin).

- **Topo-isomerase inhibitors (epipodophyllotoxins):**
These inhibitors block the enzymes topo-isomerase I and II. Topo-isomerase I and II catalyze the dent formation in the chains of DNA by which the molecule unfolds. For this reason, these agents are cell-cycle specific, with its main action in the late S and early G2-fase. The most important toxicity is myelosuppression, especially leucopenia.

- **Asparaginase:**
L-Asparaginase is an enzyme, isolated from Escherichia Coli, which catalyses the conversion of L-asparagine in L-aspartic-acid and ammonia. A lack of L-aspartic acid causes problems in the protein, RNA and DNA synthesis in leukemia cells. Side effects are depletion of fibrinogen, liver and pancreas toxicity. Also anaphylactic reactions can occur.

- **Glucocorticoids:**
These steroids inhibit inflammation through catabolic effects and stabilization of the membrane of the lysosomes. These are also immunosuppressive. Side effects are disturbances in electrolytes, osteoporosis, hyperglycemia, immunosuppressive, psychological effects, and hypercortisolism (Cushing syndrome).

- **Retinoids:**
These are derivatives of vitamin A, that include all trans-retinoic acid (ATRA), 13-cis-retinoic acid (13-cis-RA) and fenretinide. ATRA reduces (in vitro) the differentiation and proliferation of cells in hemapoietic cellines inclusive human myeloid leukemia.
cell lines and is used for the treatment of acute promyelocytic leukemia. ATRA or 13-cis-RA can cause arrest of cell growth and morphological differentiation of human NB cell lines and high dose pulse therapy with 13-cis-RA has shown to significantly improve the event free survival in high risk NB patients (after completion of intensive chemotherapy)\textsuperscript{115}. Major toxicities of 13-cis-RA are dryness of the skin and mucous membranes, conjunctivitis and hypertriglyceridemia, and of ATRA pseudotumor cerebri.

Endocrine effects of chemotherapy
The endocrine glands can be susceptible to cytotoxic agents for several reasons. Firstly, the higher the rate of cell turnover, the more susceptible an organ will be to cytotoxic agents. For example, the germ cells in males with a high rate of cell division are very susceptible to the toxic effects of chemotherapy. Secondly, a drug can target a particular biosynthetic pathway. A third factor can be the distribution of the chemotherapeutic agent and the ability of an endocrine organ to concentrate a particular drug\textsuperscript{116}. Also, the pathogenesis for endocrine complications of anti-cancer drugs may differ. Glandular dysfunction can be the direct result of cytotoxicity, or an agent can interfere with the synthesis of a hormone at one of the metabolic levels (transcription, translation or post-translation). Furthermore, an agent can interfere with the hormone and/or its receptor or with the second or third messenger, resulting in a decrease or potentiation of the interaction. Also, the carrier proteins or the binding sites on the carrier protein can be affected by the agent, resulting in altered (free) hormone plasma concentrations.

1.3.b. The effects of chemotherapy on the thyroid gland
In table 1 the reported effects of drugs on the various thyroid function determinants are shown. Next to the cytotoxic drugs, also drugs given for supportive care, e.g. anti-epileptics, diuretics or oral anti-conceptives may interfere with the thyroid function determinants. Nearly all effects described in table 1 are only present during administration of the drug. (Cytotoxic) drugs may lower or increase plasma TBG and subsequently increase or decrease plasma \( T_4 \) and \( T_3 \) concentrations, without affecting the concentration of free \( T_4 \) and TSH, and thus will not have clinical significance. Heparin may cause unreliable
### Table 1 The changes in thyroid function caused by (cytotoxic) drugs

<table>
<thead>
<tr>
<th>Effect on thyroid function</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease of plasma TSH</td>
<td>Dopamine, Glucocorticoids, Octreotide, L-Asparaginsase</td>
</tr>
<tr>
<td>Decreased thyroid hormone secretion</td>
<td>Lithium, Iodine (e.g. used for disinfectant or contrast), Amiodarone</td>
</tr>
<tr>
<td></td>
<td>Aminogluthethimide, Combinations of chemotherapy (see text)</td>
</tr>
<tr>
<td>Increased thyroid hormone secretion</td>
<td>Iodine (e.g. used for disinfectant or contrast), Amiodarone</td>
</tr>
<tr>
<td></td>
<td>Alkylating drugs (Cyclo + ifosfamide)</td>
</tr>
<tr>
<td>Increased free T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Combinations of chemotherapy (see text)</td>
</tr>
<tr>
<td>Decrease of T&lt;sub&gt;4&lt;/sub&gt; absorption</td>
<td>Colestipol, Cholestyramine, Aluminium hydroxide, Ferrous sulfate, Sucralfate</td>
</tr>
<tr>
<td>Increased TBG concentration</td>
<td>Estrogens (OAC), Tamoxifen, Heroin, Methadone, Mitotane, Fluoracil (5-Fu)</td>
</tr>
<tr>
<td>Decreased TBG concentration</td>
<td>Androgens, Anabolic steroids, Slow release nicotinic acid, Glucocorticoids</td>
</tr>
<tr>
<td></td>
<td>L-asparaginase (e.g. albumin and TBG), Combined alkylating and podophylline therapy</td>
</tr>
<tr>
<td>Displacement from protein-binding sites</td>
<td>Furosemide, Fenoclofenac, diclofenac, Mefanamic Acid, Salicylates, Heparin</td>
</tr>
<tr>
<td>Increased hepatic metabolism</td>
<td>Phenobarbital, Rifampin, Phenytoin, Carbamazepin</td>
</tr>
<tr>
<td>Decreased T&lt;sub&gt;4&lt;/sub&gt; 5'-deiodinase activity</td>
<td>Propylthiouracil, Amiodarone, Beta-adrenergic-antagonist drugs, Glucocorticoids, Radio-contrast dyes</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Interferon alfa, Interleukin-2</td>
</tr>
</tbody>
</table>

outcome of free T<sub>4</sub> measurements due to displacement of thyroid hormones from their binding sites by free fatty acids.\(^{117}\)

A transient increase of free T<sub>3</sub> has been described after the combination of 5-Fluoracil (5-FU), epirubicin and cyclophosphamide.\(^{118}\) The administration of glucocorticoids can lower TBG, decrease TSH and inhibit the peripheral conversion of T<sub>4</sub> into T<sub>3</sub>.\(^{119-123}\)
After the administration of certain combinations of chemotherapy, permanent hypothyroidism has been reported (cisplatin, bleomycin, vinblastine, etoposide and dactinomycin\textsuperscript{19}, the combination of mechlorethamine, vinblastine, procarbazine and prednisolone\textsuperscript{120}, and the combination of busulphan and cyclophosphamide (without TBI) given for conditioning before autologous bone marrow transplantation\textsuperscript{121,122}). Intra-venous administration of cyclophosphamide and ifosfamide has been reported to induce a transient increase in T\textsubscript{4} and FT\textsubscript{4} together with a fall in plasma concentration of TSH. No significant changes were found in the plasma concentrations of Tg, T\textsubscript{3}, TBG, or rT\textsubscript{3} indicating a release of thyroxine from cellular pools like the liver\textsuperscript{123}.

Cytokines probably have a direct effect on thyroid cell function and a secondary effect due to increased thyroid anti-immunity\textsuperscript{116}. After treatment with interferon-alpha, reversible hypothyroidism has been described to occur in 10-15% of treated patients. Interleukin-2 can cause an acute onset of painless thyroiditis with thyroxinemia followed by primary hypothyroidism in 20-30% of patients\textsuperscript{116,124}. Also hyperthyroidism has been described after the use of cytokines, most probably due to the development of anti-TPO antibodies\textsuperscript{116,125}.

Iodine containing drugs, like radio-contrast agents, topical iodine preparations, or ophtalmic solutions, which can release large amounts of iodine after administration, may cause hypothyroidism or hyperthyroidism, most often in patients with underlying thyroid disease and extra-thyroidal side effects due to an excess iodine (as described in section 1.1.e)\textsuperscript{22}. Most radiocontrast dyes also exert a separate effect on the thyroid gland function in that they inhibit T\textsubscript{4} to T\textsubscript{3} conversion\textsuperscript{125}. A commonly used drug in adults, also with more than just an iodide excess effect on the thyroid, is amiodarone, an anti-arrhythmic agent, which contains 75 mg of iodine per 200 mg tablet. This drug can cause thyrotoxicosis type I (mainly in patients with underlying thyroid disease), type II (destructive thyroiditis) or hypothyroidism (failure of the escape-mechanism resulting in continuous inhibition of the organification, this occurs often in presence of anti-TPO antibodies). Furthermore, amiodarone inhibits iodothyronine-5'-deiodination (D1), mediated by inhibition of cellular T\textsubscript{4} uptake, and inhibits binding of T\textsubscript{3} to its nuclear receptors (Tr\textsubscript{\alpha}, and TR\textsubscript{\beta}) by its metabolite desethylamiodarone\textsuperscript{127,128}. The effects of amiodarone can be present for months after withdrawal of the drug, most probably
due to its destructive properties on thyroid tissue, which are caused by disruption of subcellular organelle function due to binding of the drug to intralysosomal phospholipids which renders them indigestible and leads to the forming of intralysosomal multilamellar inclusion bodies 127.

The occurrence of central hypothyroidism has been reported after the administration of L-asparaginase (in combination with steroids) 129, and also the combination of vincristine, carmustine or lomustine and procarbazine increased the occurrence of permanent central hypothyroidism in adjuvant to brain irradiation 130. Furthermore, in survivors of childhood cancer, in an evaluation of “hidden” central hypothyroidism, it was found that 16 % of 62 patients with central hypothyroidism had received only chemotherapy 119.

1.3.c. The effects of chemotherapy on other endocrine organs and functions (table 2)

In table 2, the reported endocrine effects of cytotoxic agents, other than the thyroid axis, are summarized. The effects may be transient and are dose and age dependent. Also, many drugs are used in combinations, which is why the single drug effect is often uncertain.

- Gonadal damage

In table 2a the cytotoxic drugs are listed that have been associated with gonadal damage. Gonadal dysfunction caused by cytotoxic agents has been reported frequently. Damage has been described both for males and for females, mainly after the administration of alkylating agents, but also after treatment with procarbazine, nitrosoureas (carmustine or lomustine), platinum compounds, etoposide and anti-metabolites 116,131. Combinations of drugs are generally more cytotoxic than individual agents. The ovary is less vulnerable than the testis, but damage in females due to chemotherapy is not uncommon. Treatment at the age of 13 to 19 years is an important risk factor to develop ovarian damage 46. The incidence of premature ovarian dysfunction is dependent on the regimen of chemotherapy that is used; treatment with MVPP, COPP and ChIVPP resulted in 38 to 57 % of patients in ovarian failure 132. MOPP induces permanent primary hypogonadism in 12 to 46 % of women treated for Hodgkin's
Table 2. Effects of chemotherapy reported on endocrine glands, other than the thyroid

<table>
<thead>
<tr>
<th>Endocrine axis</th>
<th>Endocrine effect</th>
<th>Cytotoxic agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadal axis</td>
<td>- Sertoli cell (germ cell) damage ¹</td>
<td>Aldesleukin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Busulphan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carmustine</td>
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<tr>
<td></td>
<td></td>
<td>Chlorambucil</td>
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<td></td>
<td></td>
<td>Cisplatin</td>
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<tr>
<td></td>
<td></td>
<td>Cyclophosphamide</td>
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<tr>
<td></td>
<td></td>
<td>Cytoxizine arabinoside</td>
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<tr>
<td></td>
<td></td>
<td>Daunorubicin</td>
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<tr>
<td></td>
<td></td>
<td>Doxorubicin</td>
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<tr>
<td></td>
<td></td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Etoposide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fludarabine</td>
</tr>
<tr>
<td></td>
<td>- Leydig cell damage (increase of LH)</td>
<td>Fludarabine PO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ifosfamide ²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melphalan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVPP</td>
</tr>
<tr>
<td>- gynaecomastia</td>
<td></td>
<td>Busulphan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interferon  α₂b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitomancine</td>
</tr>
<tr>
<td>- ovarian dysfunction ¹</td>
<td></td>
<td>Adriamycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Busulphan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carmustine ¹</td>
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<tr>
<td></td>
<td></td>
<td>Chlorambucil</td>
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<tr>
<td></td>
<td></td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytoxizine arabinoside ¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doxorubicin ²</td>
</tr>
<tr>
<td>- transient amenorrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- decreased estrogen and progesterone</td>
<td></td>
<td>Cisplatin, Vinblastine</td>
</tr>
</tbody>
</table>

¹ Also combinations have been described. ¹ May have gonadal toxicity.

disease ¹³³. Due to the fact that mostly combinations of drugs are given, it is difficult to ascertain the contribution of each individual drug. However, the majority of pre-pubertal and adolescent girls that receive standard combination chemotherapy will retain or regain ovarian function. An estimated risk of 100% to develop irreversible ovarian damage has been suggested after treatment with busulphan ⁴⁶. Testicular damage concerns germinal epithelial damage resulting in oligo- or azoospermia and in Leydig cell dysfunction resulting in low-normal testosterone with elevated LH values. Germinal epithelial damage is much more frequent than Leydig cell damage, and Leydig cell failure with androgen insufficiency is relatively uncommon ⁴⁶. The available reports suggest that the chance for persistent Leydig cell damage is more likely in pre-pubertal boys than in adult males. For the germinal epithelium it is suggested,
<table>
<thead>
<tr>
<th>Endocrine axis</th>
<th>Endocrine effect</th>
<th>Cytotoxic agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal axis</td>
<td>- adrenal insufficiency</td>
<td>Busulfan (central)</td>
</tr>
<tr>
<td></td>
<td>- adrenal excess/ Cushing</td>
<td>Glucocorticoids (central)</td>
</tr>
<tr>
<td></td>
<td>- Mitotane (adrenal)</td>
<td></td>
</tr>
<tr>
<td>Gonadotropic axis</td>
<td>- GH pulsatility/deficiency</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td></td>
<td>- GH or IGF-1 resistance</td>
<td>Glucocorticoids, Daetinomycin (?)</td>
</tr>
<tr>
<td></td>
<td>- Low IGF-1 levels</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Parathyroid glands</td>
<td>- hypoparathyroidism</td>
<td>Carboplatin</td>
</tr>
<tr>
<td></td>
<td>- hyperparathyroidism</td>
<td>Cisplatin</td>
</tr>
<tr>
<td></td>
<td>- hypoparathyroidism</td>
<td>Estramustine</td>
</tr>
<tr>
<td>Mineral Metabolism</td>
<td>- Diabetes insipidus, nephrogenic</td>
<td>Ifosfamide</td>
</tr>
<tr>
<td></td>
<td>- SIADH²</td>
<td>Carbozanil</td>
</tr>
<tr>
<td></td>
<td>- Chlorambucil</td>
<td>Thiopeta</td>
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<tr>
<td></td>
<td>- Cisplatin</td>
<td>Vincristine</td>
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<tr>
<td></td>
<td>- Cyclophosphamide</td>
<td>Vinblastine</td>
</tr>
<tr>
<td></td>
<td>- Ifosfamide</td>
<td>Vindellbine</td>
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<tr>
<td></td>
<td>- Amphenicine B</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td></td>
<td>- Renal salt wasting</td>
<td>Etoposide</td>
</tr>
<tr>
<td></td>
<td>- Furosemide</td>
<td>Ifosfamide</td>
</tr>
<tr>
<td></td>
<td>- Cisplatin</td>
<td>Methotrexate</td>
</tr>
<tr>
<td></td>
<td>- Diabetes Mellitus/hyperglycemial</td>
<td>Interferon-α2a and α2b</td>
</tr>
<tr>
<td></td>
<td>- Fludarabine PO₄</td>
<td>Pentostatin</td>
</tr>
<tr>
<td></td>
<td>- Glucocorticoids</td>
<td>Streptozocin</td>
</tr>
<tr>
<td></td>
<td>- L-Asparaginase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Osteomalacia or osteoporosis</td>
<td>Estramustine</td>
</tr>
<tr>
<td></td>
<td>- Fludarabine PO₄</td>
<td>Methotrexate</td>
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<tr>
<td></td>
<td>- Glucocorticoids</td>
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<tr>
<td></td>
<td>- Mercaptopurine (6-MP)</td>
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<tr>
<td></td>
<td>- Methotrexate</td>
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</tr>
<tr>
<td></td>
<td>- Rickets</td>
<td>Ifosfamide</td>
</tr>
<tr>
<td></td>
<td>- Hypomagnesaeemia</td>
<td>Carbozanil</td>
</tr>
<tr>
<td></td>
<td>- Cisplatin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Hypocalcemia</td>
<td>Carbozanil</td>
</tr>
<tr>
<td></td>
<td>- Daetinomycin</td>
<td>Interleukin-2</td>
</tr>
<tr>
<td></td>
<td>- Cisplatin</td>
<td>Plicamycin</td>
</tr>
<tr>
<td></td>
<td>- Estramustine</td>
<td>Tumor lysis syndrome</td>
</tr>
<tr>
<td></td>
<td>- Interferon-α2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Increased phosphate</td>
<td>Estramustine</td>
</tr>
<tr>
<td></td>
<td>- Hypophosphatemia</td>
<td>Ifosfamide (tubular damage)</td>
</tr>
</tbody>
</table>

but not proven, to be the other way around; the adult testis is more susceptible than the pre-pubertal. In time, germ cell function can improve, so a normal fertility may be possible even if sustained damage has been done to the germinal epithelium \(^{131}\). For example, procarbazin containing regimes often lead to permanent infertility in males, while cisplatin based chemotherapy mostly results in temporary azoospermia \(^{132}\). It has been reported that after treatment with alkylating agents, 10-57% of males have elevated L.H levels \(^{46}\). Permanent damage is more likely after combination chemotherapy,
such as MVPP (mustine, vinblastine, procarbazine and prednisolone) and MOPP (mechlorethamine, vincristine, procarbazine and prednisolone) given for Hodgkin’s disease. The alternative regimen ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) is significantly less gonadotoxic.

Gynecomastia has been described after several cytotoxic drugs (see table 2), and has been interpreted as a manifestation of Leydig cell failure resulting in an alteration of the estrogen/androgen ratio.

- Hypothalamic damage
The direct effects of cytotoxic agents on the hypothalamus or pituitary gland have not been exactly defined yet. Some reports have been made on additional negative effects of cytotoxic drugs in addition to cranial radiation. Recently, hypothalamic damage in cancer survivors treated only with cytotoxic agents has been described; in 31 patients, 48% was diagnosed with GH deficiency, 52% with central hypothyroidism and in 32% pubertal anomalies were found.

- Growth retardation
The glucocorticoids are one of the best known drugs to cause growth retardation and are frequently administered to children during cancer treatment, as anti-emetic or as part of the cytotoxic regimen. The glucocorticoids enhance bone resorption, inhibit osteoblast activity and reduce the bone matrix production. Long term exposure to glucocorticoids interferes with GH pulsatility and decreases total GH secretion. However, normal GH and IGF-1 concentrations are found in these patients, suggesting that peripheral resistance to GH also occurs. Growth retardation caused by glucocorticoids is dose dependent and can be severe, however, these effects are transient and, in most cases, after removal of glucocorticoids a period of accelerated catch-up growth is seen.

Reduced growth velocity in children treated with multiple cytotoxic drugs is common, and it is likely that the effects of underlying disease and the different drugs interact or act additively. There are several combinations of chemotherapy that have been reported to increase the adverse reaction of growth after irradiation, such as vincristine, lomustine, cisplatin, cytosine arabinoside and methotrexate, and also in several studies
chemotherapy (multi-drug regimens, higher dose and longer duration) was the major factor attributing to growth retardation.

- Adrenal dysfunction:
The most frequent cause of adrenal dysfunction is the administration of glucocorticoids. Primary adrenal insufficiency has also been described after mitotane. Secondary adrenal insufficiency was described after busulfan in the 1960s but this has never been confirmed.116

- Disorders in the glucose metabolism:
Also with respect to insulin resistance and hyperglycemia in cancer therapy, the administration of glucocorticoids in high doses is the main cause. Furthermore, the occurrence of Diabetes Mellitus (DM) has been described after the administration of interferon-alpha, L-asparaginase and streptozocin. Drugs that can cause glucosurea without affecting glucose metabolism (renal damage) are ifosfamide and mercaptopurine. Mesna (2-mercaptoethane sulfonate sodium), which is given to prohibit hemorrhagic cystitis by ifosfamide, can give urinary ketones.116

- Disorders of water clearance:
The plasma osmolarity is normally regulated by the antidiuretic hormone (ADH), secreted by the posterior pituitary. Inappropriate ADH release (SIADH) is seen after administration of vinca-alkaloids, cyclophosphamide, cisplatin and melphalan. Renal salt wasting is described after cisplatin. Nephrogenic diabetes insipidus has been described after the administration of ifosfamide and streptozocin.116

- Disorders of mineral metabolism:
Hypocalcemia can be induced by the tumor lysis syndrome which can occur after the administration of a wide variety of cytotoxic agents (fludarabine, mitoxantrone, 6-mercaptopurine, methotrexate, chemotherapeutic regimen for BMT and various combinations). Also, without the occurrence of the tumor lysis syndrome, hypocalcemia has been reported following the administration of cisplatin, carboplatin, anti-tumor antibiotics and L-asparaginase. Osteoporosis is observed in children receiving
methotrexate, platinum compounds, glucocorticoids and several combinations of chemotherapy. Rickets has been correlated to the use of ifosfamide and estramustine.

- Disorders of the lipid metabolism:
  Hypertriglyceridemia may be caused by L-asparaginase, interferon-alpha and retinoic-acid derivatives. Hypercholesterolemia has been reported after treatment with mitotane and cisplatin. Also obesities and an increased risk for the metabolic syndrome, which is a cluster of cardiovascular risk factors including insulin resistance, glucose intolerance, dyslipidemia and hypertension, has been described in childhood cancer survivors after treatment with chemotherapy only.

1.4: Radiotherapy and endocrine effects

1.4.a. Radiation Physics
Radiation is the transfer of energy, and can be distinguished into ionizing radiation and non-ionizing radiation.
Non-ionizing radiation does not have the energy to facilitate the release of electrons from target tissues. Examples are radio waves, infra-red light and ultra violet light.
Ionizing radiation is the energy that can be released by unstable atoms to achieve stability. Ionizing radiation can have different adverse biologic effects on tissue, including damage to DNA, mRNA or proteins, by the production of free radicals, the disruption of chemical bonds, and the production of macromolecules. Radiation is used for therapy with the intention to destroy cancer cells, irreversibly, by these effects. However, it may also induce damage to healthy (surrounding) cells. As carcinogenesis can be caused by the activation or overamplification of oncogenes, or the deactivation of tumor suppressor genes or DNA repair genes, ionizing radiation can initiate carcinogenesis by introducing genetic lesions. In general, ionizing radiation is more likely to induce DNA strand breaks than point mutations. These strand breaks can lead to translocations, inversions, additions and deletions, which, if the cell does not die or the DNA is not repaired in the right way, can lead to malignancies.
Several types of ionizing radiation can be distinguished:
- Alpha particles are nuclei consisting of 2 protons and 2 neutrons. These particles
have a limited ability to penetrate intact barriers or skin, but when they are inhaled or ingested they can penetrate epithelial tissue layers to a 50 μm depth, which is enough to produce cellular injury.

- Beta particles are electrons, mostly negative (e.g. emitted from the nuclei of $^{131}\text{I}$ and $^{137}\text{Ce}$). These particles have greater penetrance than alpha-particles and can penetrate to the germinal layer of the skin. Beta emitters are most commonly used in medical applications.

- Neutrons: these are an uncommon, but very destructive type of radiation (produce 10 times more tissue damage than γ-rays), emitted only after nuclear detonation.

- X and γ-rays: both are electromagnetic radiations with wavelengths in the range $10^{-11}$ to $10^{-7}$. Gamma rays (photons) originate from atomic nuclei and X-rays originate from outside the atomic nucleus. In all other aspects these two radiation types are identical. The γ-rays are emitted from radio-active materials, like cesium-, cobalt- and iodine isotopes or after nuclear detonation, when they release energy to gain stability. X-rays are produced when high-energy particles impinge on a suitable target such as tungsten. Its electromagnetic energy varies from zero to a maximum which depends on the kinetic energy of the impinging electrons or on the material that is used as a target.

The units of activity for radiation emission for radionuclides are measured by the number of atomic disintegrations per unit time. Expressed in System Units (système international: SI): one Becquerel is one transformation per second (replaced the Curie (Ci): one Ci equals $3.7 \times 10^{10}$ transformations per second: 1 mCi=37 MBq).

The SI units of measure of energy absorbed from X- and γ-rays are the Gray (Gy) and the Sievert (Sv), which have replaced the rad (radiation absorbed dose) and the rem (rontgen equivalent man), respectively: 100 rads equals 1 Gy (=the absorption of one joule per kg) and 100 rem equals 1 Sv.

Regarding the radiosensitivity of tissue, there are several ‘laws’ that have been observed after ionizing radiation:

1. radio sensitivity increases with the rate of cell proliferation (rapidly dividing cells are more affected)
2. Radio sensitivity increases with the number of future cell divisions
3. Radio sensitivity decreases with the degree of morphologic and functional differentiation (less development the higher the radiosensitivity, e.g. chondroblasts are more radio-sensitive than chondrocytes).

1.4.6. External radiotherapy, $^{131}$I$^{-}$-treatment and $^{131}$I-MIBG

External (X-ray) radiotherapy

The most frequent used form of radiation therapy is the so-called external-beam. The energy produced by the beam governs the depth of penetration into the body. The energy is expressed in kilovoltage (kV, low energy) or megavoltage (MV, high energy). Low energy (orthovoltage) radiation was most frequently used, but has the disadvantage that it is absorbed to great extent in bone causing serious skeletal deformities. Also, in contrast to high energy irradiation, low energy has no skin sparing effect. For these reasons, nowadays high-energy radiation is most commonly used. The radiation usually comprises X-rays or photons ($\gamma$-rays) from a linear accelerator $^{141}$. The primary principle of administering radiotherapy is to give a radiation dose as high as necessary to kill a tumor, while keeping the dose to the surrounding tissues as low as possible to avoid damage to healthy tissue.

Several factors affect the likelihood and severity of radiation injury. Firstly, the higher the total dose administered, the higher the damage. Secondly, the administered dose given per fraction is of great influence on the late radiation-induced effects (the higher the dose per fraction, the higher the damage to healthy tissue). Normal tissue has a great capacity for repairing sub-lethal damage (the damage which is accumulated before a lethal effect occurs), which is impaired in malignant cells. The likelihood of adverse effects in late responding tissues is proportional to the square of the dose per fraction. This implies that when the dose per fraction is minimally increased, it can have great impact on the damaging effects even if the total dose administered remains unchanged $^{142}$. In pediatric patients it has become conventional to limit the fraction size to 1.8 Gy $^{141}$. Thirdly, the smaller the volume of tissue irradiated, the smaller the late effects will be. Examples are the craniospinal irradiation which used to be administered to patients with ependymoma, versus the local radiotherapy which is now given only to the tumor $^{141}$. Also modern radiotherapy planning techniques, the use of stereotactic radiotherapy and brachytherapy help to minimize the
volume of normal irradiated tissue adjacent to the tumor.\textsuperscript{13,14} Fourthly, the type of tissue irradiated is of importance as each tissue has its own radiosensitivity. As fifth factor, the type of radiation used is of importance; for which nowadays high-voltage irradiation is administered. Also, the use of chemotherapy during radiotherapy can potentiate the damage caused by irradiation. Examples are the use of anthracyclines and simultaneous whole lung irradiation and the development of cardiac and pulmonary toxicity.\textsuperscript{15} Finally, the age at time of radiation exposure can either increase (younger age associated with a higher risk for thyroid damage\textsuperscript{7,16}), or decrease (older age during radiation for Hodgkin's disease gives a higher risk for breast cancer\textsuperscript{141,147}) the risk to develop radiation effects.

Radio-nuclide therapy

Radio-iodide in children is administered in the treatment of differentiated thyroid carcinoma and Graves' disease.\textsuperscript{30} \textsuperscript{131}I is also used bound to organic compounds such as MIBG, to combat NB with targeted radio-therapy.

\textsuperscript{131}I emits both beta and gamma-radiation. The destruction of thyroid follicular cells is the result of the $\beta^-$-electrons. Beta particles from \textsuperscript{131}I$^-$ have a path length of 1-2 mm and will kill the cells that accumulate radio-iodine and cells in the direct environment. Histologic findings of irradiated thyroid tissue include epithelial swelling, necrosis, edema and leukocyte infiltration (see section 1.4.c). Following this acute reaction, fibrosis of the gland will follow. Administered \textsuperscript{131}I$^-$ in doses of 5.55 MBq/g thyroid tissue result in radiation doses of 120 Gy to the thyroid.

For hyperthyroidism, doses delivering 100-200 Gy are most commonly used. After treatment with 1850-3700 MBq (50-100 $\mu$Ci/g tissue), 25-50 \% of children remain hyperthyroid and after 5550-7400 MBq (150-200 $\mu$Ci/g tissue), 60-90 \% becomes hypothyroid. Due to release of thyroid hormone of degenerating follicular cells, the patient will at start become even more hyperthyroid. After 6-8 weeks, the thyroid gland shrinks and the patient will become eu- or hypothyroid. Complications of radio-iodide treatment for hyperthyroidism in children have not frequently been described. In adults, neck swelling, transient nausea, mild pain and thyroid storm have been described.\textsuperscript{30}

For differentiated thyroid carcinoma, radio-iodide is used for ablation of thyroid remnants and functioning metastases. A dose delivering 300 Gy to the residual thyroid
bed has been stated to be appropriate. Short term complications that have been described are nausea, emesis, transitory and reversible thrombocytopenia, sialadenitis and permanent decreased function of the salivary glands. Long term complications, of which concern has been raised about, are radiation-induced leukemia and secondary solid tumors in other organs that can also concentrate iodide such as the salivary glands, bladder, gastro-intestinal tract and breast. The occurrence of chronic myeloid leukemia (CML)\textsuperscript{48}, acute myeloid leukemia (AML)\textsuperscript{49} and a slight increase in the incidence of melanoma and salivary gland tumors after $^{131}$I treatment for thyroid cancer was reported\textsuperscript{150}. However, no significant increased incidence has been demonstrated for either of these diseases\textsuperscript{92,151}. It has been emphasized, though, that larger surveys to evaluate the possible long term carcinogenic effects of radio-iodine in these patients should be performed.

$^{123}$I- and $^{131}$I-MIBG

Meta-iodobenzylguanidine (MIBG), labeled with a radio-active iodine, is a compound used for diagnostic scintigraphy and therapy of neural crest derived tumors, like pheochromocytoma and neuroblastoma\textsuperscript{152}.

In the late seventies, radio-iodine labeled analogues of the adrenergic neuron blocking agent were synthesized and investigated, based on the perception of the adrenal medulla as a specialized sympathetic ganglion, with adrenomedullar chromaffin cells and adrenergic neurons showing similar catecholamine storage and secretory functions. Guanethidine is an adrenergic neuron blocking agent which bears structural similarity to the neurotransmitter and catecholamine norepinefrine. Combining the benzyl group of bretylium with the guanidine group of guanethidine led to a series of aralkylguanidines, such as MIBG, which showed an ever greater anti-adrenergic effect. MIBG is specifically taken up by the cell via the norepinephrine transporter (previously known as uptake-1 mechanism) over the cell membrane. In the cell, it is actively transported to and stored in vesicles or granules in the cytoplasm, from which it is released and becomes available for re-uptake by the same mechanism\textsuperscript{152}.

Two iodine radio-isotopes are commonly used, bound to MIBG:

- $^{123}$I emits photons with energy ideally suited for gamma cameras, whereas the minor contribution from low-energy Auger electrons results in relatively low
MIBG

absorbed doses. The convenient γ-photon energy (159 KeV) and the short half time \( t^{1/2} = 13.2 \text{ hours} \) makes \( ^{123}\text{I} \) suitable for single photon emission (computer) tomography (SPECT or SPET), which results in a more accurate quantification of photons than with planar scintigraphy.

* \( ^{123}\text{I} \) : the main γ energy of \( ^{131}\text{I}^- \) (average 364 keV) results in a lower detection efficiency than \( ^{123}\text{I}^- \), but as \( ^{131}\text{I} \) combines γ emission with cytotoxic nuclear β-emission (250-815 keV, max range 2.4 mm), this radio-isotope is widely used for both scintigraphy and therapy. The intermediate half-life of \( ^{131}\text{I}^- \) is 8.04 days.

MIBG can also be coupled to \( ^{125}\text{I} \), which has an ultra short track length with gamma-rays (35 KeV) and X-rays (27 KeV) and a relative long half-life. Due to its physical properties it can hardly be visualized, but due to its short track length it was thought to be suitable for destroying micro-metastases. However, it was not shown to have an advantageous effect over \( ^{131}\text{I}-\text{MIBG} \), and it is now primarily used for bio-distribution studies (in vitro).

\( ^{131}\text{I}-\text{MIBG} \) is used for diagnostic imaging and \( ^{131}\text{I}-\text{MIBG} \) for therapeutic purposes. About 91 % of NB takes up MIBG. Due to instability and impureness of the drug, compounds labeled with radio-isotopes can decompose, leading to the cleavage of iodide from \( ^{131}\text{I}-\text{MIBG} \). Before administration, every \( ^{131}\text{I}-\text{MIBG} \) infusion fluid for therapeutic use must be controlled for the percentage of \( ^{131}\text{I}^- \); 5 % of total radioactivity is considered as the upper limit of acceptability. In an evaluation of 17 patients, who received therapeutic \( ^{131}\text{I}-\text{MIBG} \), there was no additional forming of \( ^{131}\text{I}^- \text{ in vivo} \). However, due to differences in distribution patterns, plasma \( ^{123}\text{I} \) concentrations up to 32 % can be present after administration of \( ^{123}\text{I}-\text{MIBG} \) with only 1.7 % of free \( ^{123}\text{I}^- \).
The majority of the activity is excreted renally. After administration of $^{131}$I-MIBG the renal excretion as total radioactivity in urine is $56\% \pm 10\%$ after 24 hours, $73\% \pm 11\%$ after 48 hours, $80\% \pm 10\%$ after 72 hours and $83\% \pm 10\%$ after 96 hours. The cumulative excreted radioactivity consists for more than $85\%$ of $^{131}$I-MIBG, with $6\%$ of the dose excreted as $^{131}$I, $4\%$ as $^{131}$-meta-iodohippuric acid (MIHA) and $2.5\%$ as unknown $^{131}$I-labeled metabolites.

During administration of $^{131}$I-MIBG, patients are admitted to hospital isolation facilities for treatment with open sources (usually three-four days). Parents are instructed how to take care of the nursing of the child, to limit radiation exposure on the nursing staff. The administration of $^{131}$I-MIBG often leads directly to an improved clinical condition of the patient with NB, expressed as a relief in pain and weight gain. The main side effect of this treatment is thrombocytopenia, which can be long lasting and dose limiting. Side effects regarding the thyroid gland are discussed in section 1.4.g., chapters 2, 3, 4, and 11.

1.4.c. Radiation biology of the thyroid gland

The effects of radiation on the thyroid gland have been studied extensively, as well in animal models and in humans.

As the thyroid cells actively collect iodide, radiation experiments have, next to experiments with X-radiation, also been done with $^{131}$I. Regarding the effects of radio-iodide, it is important to recall the structure of the thyroid gland. The follicular cells, a spherical shell, surround a protein rich lumen, containing thyroglobulin. The follicular lumina occupy about 40-50% of the gland volume in rats and mice and about 50-75% in humans, dependent on the daily iodine intake. At the border of the cells and lumina, iodination takes place and the storage of thyroid hormone and iodine in thyroglobulin stored in the follicular lumina makes up about 90% of the total gland iodine. This is important to realize as it implies that most of the radio-iodine radiation emerges from the follicular spheres, possibly resulting in inhomogeneities in radio-iodine distribution.

The second important issue of influence on the radiation effect on thyroid cells (for both X-radiation as $^{131}$I), is the proliferative state of the cell, because the radiobiological response is modulated by progression through the cell cycle. In its normal state, the
thyroid gland is not in a very mitotic state \textsuperscript{160}. After stimulation by goitrogens and TSH, however, a classical growth pattern is found consisting of a lag phase, followed by a logarithmic growth and finally a plateau phase. The DNA and RNA contents of the gland increase together with the gland mass \textsuperscript{159,160}.

To study radiation effects on the thyroid, the administration of goitrogens, inducing growth of the thyroid, and the subsequent inhibition of growth after exposure to \textsuperscript{131}I has frequently been used \textsuperscript{160,161}. At radiation doses of 12-20 Gy, a reduction in goitrogenic mass increase has been described (9-15%). A further increase in dose did not result in a further growth reduction.

Also thyroid cell survival has been used to study thyroid cell radiobiology, in which the capacity of the gland to undertake cell division is evaluated, following irradiation. The survival of the thyroid cell is subsequently calculated from extrapolation of growth curves after stimulation by goitrogens or by loss of incorporated \textsuperscript{3}H-thymidine in DNA \textsuperscript{159}.

Using these above described methods, only few changes in irradiated thyroids with doses of 5-10 Gy of X-rays in experimental animals were found. Similar findings have been reported after low dose of radio-iodine (in animals with a normal iodine containing diet). At higher doses (approximately 200 Gy of radio-iodine, approaching the tissue tolerance dose) degeneration of thyroid tissue will occur in the long term \textsuperscript{160,162}. With doses of radio-iodine higher than 400 Gy, the gland will be completely destroyed. The effects on the thyroid gland induced by \textsuperscript{131}I and X-ray irradiation differ in a factor 4 to 10. These differences have mainly been contributed by the non-uniform dose distribution of \textsuperscript{131}I in the thyroid gland, difference in dose-rate, and additional factors such as dosimetry \textsuperscript{162,163}.

The biochemical consequences of thyroid irradiation, such as inhibition of purine synthesis, protein synthesis, iodide trapping and organification, are measured within a few hours after exposure to radiation. These effects can be the direct result of damage to enzymes and membranes. Already at low doses, leakage of Tg has been noted (which can be of importance in the auto-immune responses seen in the \textsuperscript{131}I\textsuperscript{-} treated patients).

The acute histological changes induced by radiation, in rats and in mankind, can be noticed within days to weeks and consist of vacuolization and eosinophilic granularity.
of follicular cell cytoplasms, pyknosis of nuclei, an increased number of phagocytes and edema of the stroma. After high doses of irradiation more severe degenerative changes are seen resulting in necrosis and desquamation of a part of the follicular epithelium. Also acute injury to the vasculature is often present, with involvement of the primary arterioles and capillaries with swollen epithelium and sometimes fibrinoid necrosis. Chronic changes include interstitial fibrosis, vascular changes, follicular atrophy, nuclear atypia (variation in size, hyperchromatism and giant bizarre nuclei), oncocytic metaplasia, lymphocytic infiltration and focal or diffuse epithelial hyperplasia.

The induction of neoplasia by radiation has extensively been studied in the rat. The two factors that are involved are firstly initiation of carcinogenesis by radiation and subsequent promotion of carcinogenesis by TSH (bovine TSH administration, administration of goitrogens or lack of dietary iodine). Thyroid glands in juvenile, neonate and fetal rats are much more radiosensitive than adult glands.

Irradiation of an unstimulated rat thyroid by X-rays or $^{131}\text{I}^{-}$ leads to neoplastic development even after small doses. However, after stimulation by TSH by the administration of goitrogens (the level of plasma TSH was not determined) the incidence of carcinoma is greatly enhanced; the incidence of adenomas increased from 40 to 98 % and of carcinomas from 0 to 23 %. Due to stimulation of TSH, an increased number of proliferating cells are found during the exposure to X-rays, making the thyroid more susceptible to radiation. In contrast, in the situation of a lack of TSH (in hypophysectomized animals), no thyroid tumors were observed, indicating that TSH is essential for tumor formation. The dose-response curve for rat radiation-induced thyroid tumors has its maximum after 100 to 150 Gy of mainly β-rays of $^{131}\text{I}$ or 5 to 11 Gy of X-rays.

As most studies are done in small rodents (rats and mice) and the pituitary gland in these animals is situated very close to the thyroid, it is important to be aware of the possibility of pituitary stray irradiation and subsequent interference on the results. It was illustrated by Walinder and Sjoden, that even under extreme care to protect the pituitary gland, the pituitary dose may still be in the order of 10-15 % of the total thyroid dose. In studies on goitrogenic growth reduction by radiation, however, it was demonstrated that accidental pituitary irradiation did not significantly affect the goitrogenic response.
1.4.d. Effects of X-radiation therapy on the thyroid gland in children

The effects of irradiation on the thyroid gland was first reported by Duffy and Fitzgerald, who discussed a group of 28 young people with thyroid cancer and noticed that 10 of them had been irradiated on the upper part of the body. Subsequently, other reports on the occurrence of thyroid carcinoma were made in children who were irradiated for benign conditions, like tinea capitis and thymus enlargement in the years 1940-1960.

In the treatment for childhood cancer, external cervical, mantle, craniospinal and total body irradiation, with the thyroid gland included in the radiation field, may be used in the treatment for Hodgkin’s disease, non-Hodgkin’s lymphoma, Acute Lymphoblastic Leukemia (ALL), naso-pharynx carcinoma, rhabdomyosarcoma, brain tumors, other tumors in the head/neck-region and preceding bone marrow transplantation (BMT). The occurrence of primary (thyroidal) hypothyroidism, subclinical hypothyroidism, benign nodules, radiation thyroiditis and Graves’ hyperthyroidism followed by hypothyroidism as well as secondary thyroid malignancies following exposure to external cervical radiation in pediatric cancer patients has been described extensively in literature. These thyroid pathologies have been described to occur in children and in adults, whereas the incidence in younger patients is higher.

Radiation-induced thyroidal hypothyroidism

The most common late effect on the thyroid gland caused by (peri) cervical irradiation is thyroidal hypothyroidism, which may be clinically overt or biochemical (subclinical or compensated) hypothyroidism. The occurrence of hypothyroidism following external irradiation has been reported to be 3 to 92%, but most frequently it is in the range of 20-30%. There is great variation in the reported data on the relationship between radiation-dose, young age, female gender and the occurrence of hypothyroidism. All these differences may be explained by different radiation techniques used, different patient-groups, different follow up times, and the different definitions that are used for hypothyroidism (biochemical or clinical).

Constine et al. have reported thyroid dysfunction in 17% of patients receiving doses < 26 Gy and 78% after doses > 26 Gy. Atahan et al. found no significant influence of the administered dose. Kaplan et al. reported that radiation with 30 Gy and
diagnostic lymphangiography both independently increase the risk to develop TSH elevation \(^{183}\). Hancock et al. did not find a higher risk for younger children; hypothyroidism was found in 15\% of children treated younger than five years of age which rose to 39\% for the children who were irradiated at ages between 15 and 20 years. However, the younger children in this study were usually irradiated with reduced doses. They concluded that age above 16 years, the female gender, the addition of chemotherapy and radiation dose were predominant factors \(^4\).

In survivors of Hodgkin’s disease, aged 2 to 20 years during RT, Sklar also found an increased risk for patients with an older age, an increasing RT dose of radiation, and for the female sex to develop hypothyroidism \(^{190}\).

To prevent or reduce the damage to surrounding normal tissue, the radiation can be given in fractions instead of a single dose. Despite this technique, still substantial endocrine morbidity has been reported, although it is less than after radiation with a single dose (16\% hypothyroidism instead of 39-59\% after ABMT) \(^{191}\). After hyperfractionated craniospinal radiotherapy in children with medulloblastoma, also a reduction of primary hypothyroidism was found (reduction from 80 to 33\%) \(^{192}\).

The adjuvant negative effect of chemotherapy on the thyroid gland was documented by Livesey et al., who reported that after the combination of mustine, vincristine/vinblastine, procarbazine, prednisolon in combination with radiotherapy a higher incidence of hypothyroidism was found than after radiation alone \(^{193}\).

As mentioned above, the use of iodinated radiographic contrast agents, especially the use of ethiodized oil emulsion in lymphangiography used in staging of pelvic and para-aortic lymphnodes and planning of radiation fields prior to external neck irradiation was thought to be a risk factor to develop hypothyroidism after RT. The explanation would be an increased TSH concentration due to the liberation of iodide and the subsequent reduction of thyroid hormone production. Fein reported that of a group of 104 patients, radiation-dose, chemotherapy or stage of disease were not of significant influence on the development of hypothyroidism, but lymphangiography was (42\% with lymphangiography against 23\% without lymphangiography) \(^{182}\). Smith demonstrated that the occurrence of hypothyroidism was influenced by the length of time-interval between lymphangiogram and radiation exposure: patients irradiated
within 10 days after lymphangiography developed most frequent or more severe hypothyroidism (mean follow-up 4.9 to 6.3 years) \(^{186}\). Also Shalet found significant greater numbers of patients with an elevated peak TSH after TRH stimulation in patients after lymphangiography and radiotherapy \(^{184}\). Tamura et al, however, did not find any increased risk for hypothyroidism after lymphangiography in 126 patients treated for Hodgkin’s or non-Hodgkin’s disease \(^{185}\). Nowadays, lymphangiography is not used anymore in these patients.

The reported time of onset of hypothyroidism (biochemically observed) varies from a TSH elevation on 6 \(^{184}\), 15 \(^{187}\), and 26 months \(^{186}\) after radiotherapy. In general, half of all thyroid dysfunctions (clinically or biochemically) will appear within 5 years, with a peak at 2-3 years, but the latency time can be as long as 20 years after radiation exposure \(^{47}\).

Next to hypothyroidism, also an eight-fold risk to develop hyperthyroidism following neck irradiation for Hodgkin’s disease has been reported, following radiation doses of 35 Gy or more \(^{190}\). The clinical behavior is comparable to Graves’ disease \(^{46}\).

X-Radiation-induced thyroid nodules and cancer

Sonographic abnormalities of the thyroid gland following radiation therapy for childhood cancers are found frequently. These abnormalities can be diffuse atrophy of the gland or focal lesions, such as single or multiple hypocchoic nodules, cysts or hyperechoic nodules.

The incidence of thyroid nodules after radiation has been described from being 2-87% \(^{46,190,196,197}\), with, in survivors of Hodgkin’s disease, a relative risk (RR) of 27 \(^{190}\). The female sex and a radiation dose to the thyroid of 25 Gy are independent risk factors for the development of nodules \(^{190}\). The plasma concentration of Tg correlates to the number but not to the volume of the thyroid nodules \(^{197}\). Also the younger age at radiotherapy, the longer follow-up and the length of time that TSH is elevated are associated with an increased occurrence of thyroid nodules \(^{181,196,198}\).

With the improving techniques of ultrasonography more thyroid nodules will be found than after palpation, also after exposure to radiation \(^{197}\). The advantage is that potentially malignant nodules can be detected in an early stage, the disadvantage is the finding of many benign, and perhaps clinically non-relevant smaller nodules (incidentaloma’s). As the recurrence of nodules has been described to decrease by TSH suppression
and, after radiation, the risk of a nodule to develop into a malignancy is increased, it is advised to administer T_4 in case of nodules after exposure to irradiation, although there is no real evidence to support this \(^{16}\). In a cohort of 46 patients after X-RT for Hodgkin’s disease, of which 34 did not receive T_4 and 12 did receive T_4 administration for an elevated TSH, 14/34 developed a focal lesion, while in 4/12 patients resolution of a focal lesion was with T_4. However, also 4 patients showed increase of nodules and three developed new lesions during T_4 administration \(^{196}\). More studies regarding T_4-therapy to prevent thyroid nodules and carcinomas after irradiation are discussed in Chapter 11.

To gain more information on histological level, fine needle aspiration cytology can be done. This is advised in nodules of 10-15 mm or larger \(^{197}\).

The reported incidence of thyroid carcinoma after treatment for childhood cancer varies enormously in literature, which is due to differences in age at radiation, dose of radiation and the length in follow-up. Of all patients diagnosed with a thyroid malignancy, around 10-40 % has been exposed to irradiation \(^{199}\). In a pooled analysis of 7 studies, for patients exposed to irradiation for benign diseases before the age of 15 years, a linear dose-response was found down to 0.1 Gy, but beyond 10 Gy a decrease or leveling of risk was seen. The excess absolute risk (EAR) was 4.4 cancers/10,000 person-years Gy and the excess relative risk (ERR) was 7.7/Gy, for females greater than for males. In this series, only 2 cases were seen within 5 years after radiation exposure \(^{78}\). The ERR for children who are irradiated for malignant diseases to develop radiation-induced thyroid cancer may be different, probably dependent on a genetic predisposition to develop cancer. In 9170 survivors of childhood cancer, a 53-fold increased risk to develop thyroid cancer has been found, increasing in time. Radiation doses greater than 2 Gy increased the risk for thyroid cancer 13 times, and the risk did not decrease at a radiation dose of even 60 Gy \(^{146}\). The latency time for the development of thyroid cancer has been reported to be as long as 30 years and the ERR is still elevated after 40 years \(^{78,198}\).

Among children, an increased risk to develop thyroid tumors after irradiation is found for females. Also the use of dactinomycin has been associated with an increased risk \(^{188}\). Furthermore, an increased susceptibility to develop thyroid tumors has been suggested for children with NB. For children with NB the occurrence of thyroid tumors
(carcinomas and adenomas) after irradiation was five times more frequent than for other childhood malignancies, which could not be explained by age, sex, dose or length of follow-up \(^{31}\). Also, in a hospital-based study by Tucker, after correction of radiation dose, a rate of 2.1 thyroid cancers per person-years/cGy was found for survivors of NB compared to 1.6 for Wilms' and 0.3 for Hodgkin's and non-Hodgkin's lymphoma \(^{146}\). In a third study, 5 thyroid cancers were found in 544 5-year survival NB patients, all after receiving external radiotherapy, with a corresponding average ERR of 0.50 per Gy \(^{201}\). Biological arguments explaining this increased susceptibility might be the fact that tumors of the sympathetic nervous system can be related to MTC and that in MTC and differentiated thyroid tumors similar chromosomal deletions or abnormalities are found (chromosome 10 q arm) \(^{146}\).

Most X-radiation-induced thyroid carcinomas are papillary (85 %), but also follicular tumors (10%) and medullary or undifferentiated carcinomas occur \(^{50}\). A higher prevalence of RET/PTC arrangements has been found in radiation induced PTC compared to sporadic PTC \(^{202,203}\), with, in contrast to the Chernobyl tumors, a higher frequency of the chimeric gene RET/PTC1 instead of RET/PTC3. However, in a recent performed evaluation of tumors occurring after low dose external radiation an overall RET/PTC activation of 38.6 % was found in all papillary carcinoma, with no association to radiation exposure \(^{204}\).

There is no difference in behavior or prognosis for patients with radio-induced or non-radio induced thyroid cancer \(^{46}\). Ways to protect the thyroid from X-radiation induced damage are discussed in section 1.4.f., chapter 6 and chapter 11.

1.4.e. Effects of \(^{131}\text{I}^-\) on the thyroid gland in children
Thyroid damage due to radio-iodide has been extensively reported following the Chernobyl nuclear disaster. The accident in this power plant on April 26\(^{th}\), 1986, resulted in the release of about 444 \(10^7\) GBq of radionuclides, containing \(^{239}\text{Pu}\), \(^{240}\text{Pu}\), \(^{137}\text{Cs}\) and about 15-20 \% \(^{131}\text{I}^-\)iodine and 30\% \(^{131}\text{I}^-\)iodine \(^{138,205}\). In the children living in this region, and also in children exposed to radio-iodine isotopes in the Marshall Islands, an increase in the occurrence of benign thyroid nodules and thyroid cancer has been described \(^{206,206}\).
The nodules, that arise after $^{131}$I-exposure, are predominantly adenomatous, more well-encapsulated with papillary patterns and degenerative changes, compared to those found after external irradiation. In the nodules of the Chernobyl children, a significant higher number of RET/PTC rearrangements were found (52.4%) compared to naturally occurring nodules (13.9%), but not when compared to externally irradiated nodules (37.5%).

The children under 5 years of age were especially at risk to develop thyroid carcinoma. At first there was concern that there might have been an ascertainment bias, but the numbers of affected children exceeded an unprecedented incidence, which increased from less than 1 per million per year to more than $30 \times 10^6$ per year (Belarus) and more than $3/10^6$ per year (Ukraine). For children and young adolescents, in iodine deficient areas, the risk to develop thyroid carcinoma was twice as high. For adults, very recently also an accelerated increase of thyroid cancer was reported in all age categories from 1990 onwards. The thyroid carcinomas that have been found in children in the "Chernobyl-regions" are characterized by a short latency period and an almost equal sex ratio. The tumors were often aggressive, predominantly papillary, with intraglandular tumor dissemination in 92%, thyroid capsular and soft-tissue invasion in 89% and cervical lymph node metastases in 88%, with a short latency period between exposure and disease.

A very high frequency (95%) of RET/PTC rearrangements was found (PTC 1 to 8). In the tumors with a short latency period (<10 years) more frequent RET/PTC3 rearrangements are found and in late occurring tumors more frequent RET/PTC1 rearrangements. However, a study performed in 2001 could not confirm the differences in RET/PTC1 or RET/PTC3 rearrangements, and could also not confirm the differences in frequency of RET-rearrangements between irradiated tumors and sporadic, or between adults and younger children, indicating that other factors may act after irradiation exposure that leads to RET proto-oncogene activation. Ras and p53 mutations do not seem to play a role in the thyroid carcinomas that are found in the "Chernobyl-region".

The chance to develop thyroid nodules and carcinoma after medical use of radioiodine is unclear. There is no evidence that diagnostic use of $^{131}$I, with a dose of 2.22 MBq resulting in an estimated dose of 6.5 Gy to the thyroid, increases the risk for
thyroid carcinoma \(^{30,214}\). Also, the therapeutic use of \(^{131}\)I for hyperthyroidism or \(^{131}\)I use in cardiac disease has not led to an increased rate of thyroid cancer or thyroid cancer mortality.\(^{215,216}\) All these results apply for adults, however, and follow-up data on children given diagnostic \(^{131}\)I is scarce. In children treated with radio-iodide for hyperthyroidism, the reported occurrence of thyroid nodules ranges from 0 to 30% (after 1.85 MBq/g tissue). No increased risk has been reported for children to develop thyroid malignancies \(^{50}\), which can be explained by the fact that cell-killing radiation doses are used. The very few reports on thyroid cancer following radio-iodide in children with hyperthyroidism were associated with the use of low-moderate doses of radio-iodide (1.85 MBq/g and 46.25 to 199.8 MBq)\(^{50}\). However, considering the relative small numbers of children, the short follow-up time and latency time of thyroid tumors following external radiation, follow-up of children given diagnostic and/or therapeutic \(^{131}\)I is still warranted.

In the treatment for differentiated thyroid carcinoma, the radio-iodide will ablate all the remains of thyroid tissue after thyroid surgery, resulting in hypothyroidism. \(T_4\) administration is required for thyroid hormone substitution and subsequent TSH suppression.

1.4.f. Prevention of radiation damage to the thyroid gland

Considering all above described adverse effects of radiation on the thyroid gland or its function, ways to protect the thyroid in children who need radiation treatment are necessary. Ways to protect the thyroid gland against radiation can be 'thyroidal' interventions or 'general radio-protective' interventions. Examples of thyroidal ways of prevention are the suppression of TSH and inhibition of NIS, examples of general radio-protective ways are the administration of free radical scavengers.

As shown in figure 7, protection of the thyroid against external radiation (XR) or radio-iodine can be attempted by interfering on different levels: at the level of the pituitary gland (decreasing the plasma concentration of TSH and/or GH), at the level of the circulation (decreasing the vascularization/oxygenation and decreasing the exposure to radio-iodine), at the level of the active transport of iodide into the thyroid (NIS) (decreasing uptake of radio-iodide), at the site of organification (by TPO)
Figure 7. Possible ways of protection against radiation-induced thyroid damage

Schematic figure for the iodine pathway in the thyroid follicle and the possible ways of radiation prevention. After stimulation of TSH, iodide is actively taken up via the sodium-iodide transporter (NIS). Subsequently it is transported to the apical membrane, where it is enzymatically (TPO) oxidized by peroxide into hypoiodite and subsequently coupled to and bound into thyroglobulin (Tg). After stimulation by TSH, Tg is endocytosed, lysosomely hydrolyzed and MIT, DIT are deiodinated and T4 and T3 are secreted into the circulation. When the intra-thyroidal concentration of iodide is high, the compound XI is formed reducing a.o. NIS activity and cAMP formation. 1=151I- decreases the concentration of 131I-, decreases the vascularization of the thyroid, decreases NIS activity, decreases organification, possibly stimulates the forming of compound XI which decreases cAMP formation but catalyses the generation of O2 radicals.
2=ClO2- decreases 131I- uptake by NIS. 3=PTU/MMI decrease iodide organification and decrease the forming of free radicals. 4=selenium (animal models) decreases the forming of free radicals, 5=Thyroid hormone decreases 131I- uptake by NIS and cAMP formation by decreasing TSH, 6=ACTH/cortisone (animal models), decreases radio-iodide uptake by decrease of TSH concentration. 7=gonadal hormones increase TSH concentration, 8=SCN- decreases NIS mediated uptake of 131I, 9=hypophysectomy (animal models) decreases plasma TSH and the concentration of GH (proliferation thyroid cells). 10= excretion of 131I by the kidneys (increasing efflux of radio-iodine and decreasing the amount of free radicals), and at the site of endocytosis into the cell or as T4 & T3 into the circulation.

Because there are several differences between the possible ways of protection against radio-iodine or XR, these noxes will firstly be discussed separately. Subsequently, regarding the similar possibilities of preventive intervention, they will be discussed together.
Prevention of thyroid radiation damage due to radio-iodine

Without protection, the thyroid will accumulate about 20-40 % of administered radio-iodine, dependent on the thyroid's functional status and the iodine status. Because the risk to develop thyroid malignancies after exposure to radiation has been shown to be already increased after exposure to 0.1 Gy \(^{78}\), prevention of uptake of radio-iodine in children must be aimed to reduce uptake to 0%. Next to a reduction to 0 % uptake, any preventive strategy should preferably have a minimum of side effects.

Reduction of exposure to radio-iodine by interference on the pituitary level indicates lowering TSH, because TSH stimulates iodide uptake into the thyroid, iodide organification and accumulation, thyroid hormone formation, Tg endocytosis and proteolysis and release of thyroid hormones \(^{10,16}\). Lowering TSH can be achieved by hypophysectomy (animals \(^{21}\)), the administration of a TSH antagonist (developed for the treatment of TSH producing adenoma \(^{218}\), but not yet used for radiation protection) or the administration of \(T_4\) or \(T_3\).

Protection of the thyroid against radio-iodine exposure has been done most frequently by interference at the level of the circulation: the administration of stable iodine \((^{125}I)\), mainly using potassium iodide (KI). The administration of high doses of stable iodine dilutes or saturates the amount of radio-iodide in the circulation, resulting in a diminished exposure to radio-iodine. Also, high concentrations of iodine will acutely diminish the vascularization of the thyroid, which results in a decreased exposure to extra-thyroidal radio-iodide.

Furthermore, the administration of KI (or any other iodine solution) has its effect on several other levels, as explained in section 1.1.c. Whether a decreased activity of NIS caused by the escape mechanism of the Wolff-Chaikoff effect contributes to the decrease in uptake of radio-iodide as radio-protective effect is unclear \(^{219}\). It has been shown in animals that also during exposure to KI, euthyroidism is rendered \(^{230}\), with only a slight increase in TSH. This implies that active iodide transport by NIS is present during KI administration and also active uptake of radio-iodide remains possible. A second argument against the involvement of the Wolff-Chaikoff effect in radio-protection of excess iodide is the fact that reduction in uptake, in rat thyroids, is already observed at iodide plasma levels or doses where organic iodine is still increasing \(^{219}\). However, the ability to concentrate iodide has been shown to be a function of the
iodine content of the gland, indicating that the iodide transport mechanism is dependent on the amount of glandular iodine and not to saturation of the circulation alone. The maximal reduction in uptake is achieved when KI is administered briefly (at best one hour) before radio-iodine exposure. When KI is administered much earlier before exposure, it will lead to a much less efficient reduction in uptake. Another effect of a high concentration of (cold) iodine possibly contributing to a radio-protective effect is a decrease in cAMP leading to a decreased metabolic activity of the cell.

Side effects of iodine should always be taken into consideration, when administered for thyroid protection, which may be thyroidal, i.e. iodide goiter with or without hypothyroidism, hyperthyroidism and hypothyroidism (most important in neonates and possibly also in young children and the elderly when the escape mechanism of the Wolff-Chaikoff effect fails), or non-thyroidal, i.e. dermatological and mucous reactions, iodide mumps, serum sickness (hypersensitivity) and vascular reactions. Most side effects are transient. The FDA has recommended that KI is safe to administer as thyroid protection in case of nuclear accidents. The recommended dose is 130 mg for adults and 65 mg for children below one year per day when they are likely to receive a projected dose of 25 rem or greater from radio-iodines released into the environment. KI is also often administered for thyroid protection in case of use of 131I for medical applications, its effectiveness will be evaluated in chapters 2, 3, and 11.

As described in section 1.1.b, the inhibition of NIS, next to iodide, can also be done by the administration of other anions. From studies in children with a total iodide organification defect, who are given the CLO₄⁻-discharge test, it is known that, if given in the appropriate dose, CLO₄⁻ leads to a 100% inhibition of radio-iodide uptake leading to 0% uptake after an hour. However, CLO₄⁻ has been associated with more serious side effects than KI. CLO₄⁻ has been shown to suppress bone marrow function and in the 60's, 7 cases were reported with fatal aplastic anemia, after administration of high doses of CLO₄⁻ for 2-6 months. For this reason, it is now mainly used as single dose for diagnostic purposes. However, in lower doses it is also used in the treatment of amiodarone induced hyperthyroidism and no serious side effects or additional cases of aplastic anemia have been reported.

Intervention on the level of the thyroid follicular lumen, the organification by TPO, can be done by the administration of anti-thyroid drugs, which inhibit the organification
and lead to a faster efflux of radio-iodide. Also, as explained previously, when the intra-thyroidal concentration of iodide is elevated it inhibits its own organification by reduction of the $H_2O_2$ generation (ThOX and TPO). $CLO_4^-$ has very little or no effect on the organification. Intervention on the level of endocytosis and secretion of thyroid hormone can be done by the administration of TSH, which will increase the elimination of radio-iodide as it speeds thyroid hormone formation, Tg endocytosis, lysosomal hydrolysis and the release of thyroid hormones. Stimulating the elimination of radio-iodine by the kidneys, which is the main way to excrete (radio-active) iodine, to reduce internal radiation exposure can be done by stimulating diuresis.

Protective strategies against X-irradiation (XR)

"Physical ways of prevention" that are undertaken and that have shown to be protective against the effects of XR are elimination of radiation exposure (by applying lead shields or replacing radiotherapy with chemotherapy), reduction of total dose, and hyperfractionation. Before applying these preventive strategies though, it must be guaranteed that the efficacy of the treatment for the malignant disease remains just as good.

With regard to the replacement of radiation with chemotherapy, the possible negative effects of chemotherapy must be carefully outweighed, as for example hypothyroidism or even thyroid carcinoma caused by cervical irradiation may be easier to treat than infertility caused by alkylating drugs.

Protective strategies against both radio-iodine and XR

For both exposure to radio-iodine and exposure to XR, protection for the thyroid gland is thought to be brought about by lowering of TSH during exposure to radiation as the level of TSH is of direct influence on the (mitotic) activity of the thyroid cell. The effectiveness of lowering TSH during and after exposure to radiation in animals and humans is discussed in chapters 6 and 11.

Inhibition of iodide organification by anti-thyroid drugs may, besides a reduced binding of radio-iodine, also reduce the hydrogen peroxide concentration by a still unknown regulation mechanism. A diminished oxidation and generation of $H_2O_2$ will subsequently decrease the production of free radicals. The production of free radicals
is one of the main factors involved in cellular damage due to radiation exposure by breaking chemical bonds, disruption of the membrane structure, DNA mutation and structural damage to proteins. Scavenging these radicals will decrease cellular radiation damage. Amifostine, an aminothiol known as WR-1065, has been shown to protect the salivary glands during X-radiation based on free radical scavenging and possibly by genomic stabilization \(^{221}\). A trial using amifostine for thyroid protection has been suggested, but up to now no results have been published \(^{224}\). However, a note must be made, that amifostine has also recently been associated with a high percentage of adverse effects \(^{225}\).

Natural occurring anti-oxidants are radioprotective. Sulphydryl compounds, such as cysteine (precursor of glutathione), glutathione and β-mercaptoethylamine (cysteamine), can be considered anti-oxidants and may protect cellular DNA by several mechanisms, including free radical scavenging, hydrogen donation, and modulation of repair processes \(^{223}\). Furthermore, radiation exposure may alter the balance of endogenous protective systems such as glutathione and antioxidant enzyme systems and it is probable that antioxidant enzymes, such as glutathione peroxidase, manganese superoxide dismutase, copper-zinc superoxide dismutase and catalase provide radiation protection. However, the right balance in which these enzymes are required is far from clear \(^{223}\). Antioxidant nutrients, such as selenium and several vitamins (vitamin A, C, E and glutathione) are known to act as radioprotectors \(^{223}\). Selenium and vitamin E, may not only be antioxidants but also have other physiological activities by which they modulate radiation responses, however these mechanisms are not completely clear \(^{223}\). It has been shown that cellular pretreatment with selenium, which is a component of glutathione peroxidase, results in increased levels of glutathione peroxidase, catalase, and nonprotein thiols (glutathione) and in an enhanced destruction of peroxides \(^{226}\). Moreover, selenium has a special effect on thyroid hormone metabolism as it is required for all 3 iodothyronine deiodinases, a.o. necessary for the conversion of T\(_4\) into T\(_3\). These enzymes contain selenocysteine in the catalytic side. Selenium deficiency is therefore thought to exacerbate hypothyroidism in iodine deficient regions \(^{22}\).

Other anti-oxidants, that have shown to be radio-protective are the endogenous metals ions as from zinc, iron, copper, magnesium and phytochemicals, such as flavonoids,
methylxanthines (caffeine and theophylline) and melatonin. Regarding the administration of non-thyroid specific anti-oxidants to children with malignant diseases it must be guarded that the efficacy of cancer treatment is not affected. The effectiveness of preventive strategies for thyroid damage due to radio-iodine and XR in humans and animals is further discussed in chapters 2, 3, 4, 6 and 11.

1.4.g. Prevention of thyroid damage due to $^{131}$I-MIBG in children

In the treatment for neuroblastoma, radio-MIBG is administered, 2-4 times, in doses ranging from 3.7-7.4 GBq. During administration of $^{131}$I-MIBG, $^{131}$I$^-$ is released intra-venously, due to instability of the drug (2-5%) and another small percentage, which is cleaved off by liver metabolism.

Until 1999, in the Emma Children's Hospital, AMC, to prevent the thyroid from uptake of radio-active $^{131}$I$^-$ during MIBG treatment, patients were given 100 mg KI daily for two weeks during treatment with $^{131}$I-MIBG, and for 5 days during diagnostic $^{123}$I-MIBG. The thyroid glands from parents is protected by 200 mg KI daily during three days.

The effect of radio-iodide after treatment with $^{131}$I-MIBG on the thyroid gland, despite protection with KI, has been reported by Picco et al. In 14 long term surviving patients with neuroblastoma, treated with $^{131}$I-MIBG, surgery and conventional pharmacologic therapy, 12 developed a diminished thyroid function. KI was administered orally from 7 days before until 7 days after $^{131}$I-MIBG administration. 2-3 mg iodide per kg body mass was given, once daily, and an additional dose was given if the patient experienced emesis within three hours. Within 6 months, 5 patients showed thyroid abnormalities, of which 2 overt hypothyroidism. Within 12 months, 6 patients were diagnosed with high TSH, but $T_4$ and $T_3$ levels were within the normal range (compensated hypothyroidism). In two years, 8 of the 14 patients developed overt primary hypothyroidism, presenting with thyroid enlargement and typical hypothyroidism-related symptoms including, in 2 patients, a decreased growth velocity. All of these 8 patients required replacement treatment with L-thyroxine (mean dose 3 $\mu$g/kg per day). Four other patients developed asymptomatic elevated TSH concentrations. Three patients treated with radio-MIBG and total body irradiation (TBI) developed overt hypothyroidism.
In a report on second malignancies in 119 children with NB after combined treatment including $^{131}$I-MIBG, with a mean follow-up time of 1.5 years (range 0.1-16.3), 5 malignancies were found (myeloid leukemia (2), angiomatous fibrous histiocytoma, malignant schwannoma, rhabdomyosarcoma), but no thyroid malignancies.

These results indicate that oral administration of KI seems insufficient for protection of the thyroid function during treatment with radio-MIBG, which may result in hypothyroidism. No increased risk for the development of thyroid tumors has been reported though. Considering the relative short follow-up, this should be studied in larger cohorts over a longer follow-up time.

Protection of the thyroid gland during MIBG-treatment will be further discussed in chapters 2, 3, 4 and 11.

1.4.h. Effects of X-radiation therapy on other endocrine organs

Hypothalamic-pituitary gland

Hypothalamic-pituitary dysfunction following radiation therapy is, like the thyroid gland, time and dose dependent. The incidence of problems increases in time. For both the hypothalamus and the pituitary gland, the dose per fraction that is administered is of importance next to the total administered dose. Younger patients are more vulnerable than older patients.

The hypothalamus is more radiosensitive than the pituitary gland; damage can be expected after 40-50 Gy, and after higher doses also the pituitary gland may be damaged. However, also at doses < 40 Gy hypopituitarism may be present due to hypothalamic dysfunction. Of the production of the pituitary hormones, the synthesis of GH will be affected first, mostly followed by LH & FSH, ACTH, and TSH. A GH deficiency can already occur after irradiation with 18 Gy. Dose and time from irradiation are significantly correlated to GH deficiency, but the speed of onset in the first five years is dose dependent. After cranial irradiation with 27 Gy or more, GH deficiency will be manifest within 2 to 5 years. Next to GH deficiency, also GH neurosecretory dysfunction has been described to occur, characterized by a normal GH peak in the stimulation test but a decreased spontaneous 24-hour GH-secretion. Neurosecretory GH dysfunction has been described after short, relatively high RT doses (10 Gy in 3 days) for BMT.

Gonadotropin deficiency is frequent after cranial RT for a brain tumor, especially
after radiation doses of \( > 50 \text{ Gy} \) \cite{231}. In contrast, after lower doses (18-47.5 Gy), precocious puberty is associated to radiation exposure, mainly for girls \cite{234, 235}, explained by a disinhibition of cortical influences on the hypothalamus which increases the frequency and amplitude of GnRH secretion \cite{135}.

ACTH deficiency is rare following low dose irradiation, but its frequency increases after exposure to more than 50 Gy. In patients with naso-pharyngeal carcinoma, ACTH deficiency was present in 27 \% of patients after 5 years \cite{233}. However, subtle abnormalities may be present in this axis, which should be addressed with caution when using this axis in times of stress. Also, the adrenal androgen production may be distorted by cranial irradiation, detected by subnormal DHEAS concentration as a result of diminished ACTH production \cite{233}.

In contrast to the decrease in production of most pituitary hormones following cranial irradiation, the levels of prolactin are often increased \cite{135, 231}, occurring in approximately 20-50 \% of patients after RT \cite{116}. The increase of PRL is thought to be caused by loss of the normal inhibition of PRL secretion due to damage to the hypothalamus. Hyperprolactinemia causes inhibition of the gonadotropin secretion and decreases the sensitivity of the pituitary gland to GnRH, causing hypogonadotrophic hypogonadism.

Hypothalamic radiation damage may lead to obesity (most often after radiation doses \( > 51 \text{ Gy} \)) and the metabolic syndrome, for example following treatment for ALL or brain tumors. This hypothalamic obesity has been related to post-cranial irradiation leptin resistance leading to the inability to recognize satiety and to vagally mediated hyperinsulinism caused by destruction of the ventromedial hypothalamus (VMH) \cite{46, 236}.

Many other factors may also contribute to this syndrome, including increased energy consumption, decreased energy expenditure and hormonal disbalance including GH deficiency, hypothyroidism and hypogonadism \cite{237}. Treatment with octreotide may be beneficial for pediatric patients with hypothalamic obesity \cite{236}.

Central hypothyroidism

The occurrence of central hypothyroidism has been described in 39 \% of patients after cranial radiation with 30 Gy or more and in 8 \% after 15-29 Gy \cite{110}. Also, irradiation of the pituitary gland has been described to reduce the biological activity of TSH \cite{238}.

Chemotherapy has been documented to increase the occurrence of central
hypothyroidism as mentioned above. Mixed hypothyroidism has also been described in survivors of childhood cancer, and can be diagnosed by finding a mild elevation of TSH with low levels of free T₄. In this syndrome, TSH might be abnormally glycosylated due to reduced TRH release from the hypothalamus. These findings may represent the combination of thyroid and pituitary/hypothalamic damage or central hypothyroidism (hypothalamic damage), and is most prevalent after cranial or cranio-spinal irradiation with > 30 Gy.

Gonads
The gonads are highly radio-sensitive. In males, the testicular germ cells, due to the high rate of cell division, are more sensitive than the Leydig cells or Sertoli cells. After radiation with 0.1 Gy, morphologic and quantitative changes in spermatogonia are observed. Doses above 0.8 Gy result in azoospermia and below 0.8 Gy in oligospermia. Recovery may take place within 9-18 months after 1 Gy, within 30 months after 2-3 Gy and after 5 years after doses above 4 Gy. A rise of LH (due to damage in Leydig cells), as a sign of decreased testosterone production, has been demonstrated after radiation doses above 0.75 Gy and fractionated doses above 2 Gy.

In girls, because oogenesis occurs during embryonic life, the oocytes are in a relative quiescent state and are much more radioresistant. However, when they are damaged, they can not be replaced or repaired. The radiosensitivity of ovarian tissue is dose and age-dependent, and the LD₅₀ of the human oocytes (representing the dose of radiation required to destroy 50 % of the primordial follicles) has been calculated to be < 2 Gy. In a cohort of 19 girls treated with radiotherapy for intra-abdominal malignancies during childhood, 18 developed ovarian failure after exposure to 30 Gy (16-26 fractions). In a cohort of 38 patients who received whole abdominal X-RT (20-30 Gy), 27 failed to undergo or complete pubertal development and 10 underwent premature menopause (median age 23.5 years). Of 15 patients who received flank X-RT with the same dose, ovarian function was normal in all but one in whom pubertal failure occurred. In this cohort no live births were achieved, with all miscarriages occurring in the second trimester.
Parathyroid glands

Hyperparathyroidism has a 2.5 to 3 fold increased occurrence after low-dose head neck irradiation (2-7.5 Gy), but after higher doses it is relatively uncommon. In a review performed on 2556 patients, the relative risk to develop hyperparathyroidism after head-neck irradiation was found to be 0.11 per cGy, with a wide confidence interval (0.0-17.2), and a latency time of 25 to 47 years. Despite this increased risk, hyperparathyroidism is not often diagnosed after external irradiation. Reason for this probably lies in the fact that it often remains asymptomatic and thus escapes clinical detection.

Bones

Next to a radiation-induced GH-deficiency, growth retardation in children irradiated for a childhood malignancy can also be explained by direct radiation damage to the long bones or the spine (due to (cranio)-spinal irradiation or total body irradiation), resulting in skeletal disproportion. For this reason it is important to measure both total length and length of the spine (sitting height). Furthermore, the simultaneous presence of other central endocrinopathies, like hypothyroidism and premature puberty, may increase the growth retardation.

1.4.i. Effects of $^{131}\text{I}$ on other endocrine glands in children

After treatment with radio-iodide, it has been suspected that the gonads may be damaged. In male patients an increase in FSH is described but not in LH, suggesting damage to the germinal cells. In female patients the fertility rate after radio-iodide treatment for thyroid carcinoma, was not affected, although various reports have been published of increased rates of miscarriages. No significant increased risk for leukemia or other solid tumors has been found after radio-iodide treatment, although reports have been written of increased cancer in organs that concentrate $^{131}\text{I}$ (breast, salivary glands, digestive tract, kidney and bladder). More studies on secondary cancer following radio-iodide treatment are necessary.
1.5: Surgery and endocrine effects in children with cancer

The endocrine complications of a surgical procedure are, of course, dependent on the localization of the tumor, the size and the ingrowth in surrounding tissue. After surgery for craniopharyngioma, next to panhypopituitarism, obesity related to hypothalamic injury can be a very distressing outcome. Recently, it has been shown that in this patient group many features of the metabolic syndrome are present. After surgery for NB, endocrine effects might be expected after performing adrenalectomy. However, no cases of hypocortisolism have been reported following adrenal surgery. In chapter 3, the effects of surgery on plasma cortisol and ACTH are discussed.

After total thyroidectomy for thyroid carcinoma, an (intended) endocrine effect is the development of hypothyroidism. Treatment can adequately be given by the supplementation of T₄. The main postoperative complications are laryngeal palsy and hypoparathyroidism. With regard to the risk of hypoparathyroidism, special attention in the post-operative period should be paid to the development of hypocalcemia due to the lack of parathyroid hormone (PTH). Substitution therapy can be given as calcium and vitamin D. The occurrence of these side effects is discussed in more detail in chapter 7.

Other endocrine effects of surgery after treatment for pediatric cancer may be hormonal deficiencies following removal of brain tumors near the hypothalamus and pituitary region, and gonadal tumors (ovarian cysts, gonadoblastoma and testis carcinoma).

1.6: The phenomenon of ‘non-thyroidal illness’ in children with cancer

During caloric deprivation or severe illness, the concentration of plasma T₃ decreases while the concentration of plasma rT₃ increases and the concentration of plasma TSH is normal or low. Total T₄ may remain in the normal range or may be lowered. This phenomenon has been called the syndrome of non-thyroidal illness (NTI) or the euthyroid sick syndrome. In adults, this syndrome has been described in a wide variety of conditions: severe infections, trauma, myocardial infarction, major surgery, malignancy, inflammatory conditions, starvation or can be caused by medication (e.g. dopamine).

In children, abnormalities of the thyroid hormone determinants have been described in
prematures, in neonates in combination with the respiratory distress syndrome, in critically ill children in the intensive care unit (a.o. after cardiac surgery or meningococcal sepsis), in badly regulated diabetes mellitus and hepatitis A.

Five conceptual interpretations for NTI are given in literature: 1) the abnormalities represent test artifacts, 2) the serum thyroid hormone abnormalities are due to inhibitors of T₄ binding proteins, 3) the pituitary is euthyroid and the body is hypothyroid, because pituitary T₃ levels are normal, 4) patients are biochemically hypothyroid but this is a beneficial physiological response to illness and should not be altered by treatment and 5) the patient is actually hypothyroid and this is probably disadvantageous to the patient.

The exact underlying cause of this changed thyroid hormone state has not been defined yet, although several factors have been shown to play a role: a diminished conversion of T₄ to T₃ due to a decrease of type 1-iodothyronine deiodinase, an altered function of the hypothalamus and pituitary gland, an increase of stress-induced glucocorticoids and the administration of medication. The role of circulating cytokines has not been completely identified yet. It has been shown that IL-6, Interleukin-1, II-aRA and other factors are correlated to the suppression of thyroid hormone levels, but neutralization of the effects of cytokines (by the use of receptor antagonists or blocking antibodies) did not support the evidence for NTI. Also the administration of cytokines (II-1, II-6, TNF and IFN) to healthy volunteers did not result in NTI. However, it has been demonstrated that NTI is 'an acute phase response' which is generated by activation of a cytokine network. For this reason, it may be the combination of cytokines that work via the hypothalamus that result in suppression of TRH production. Also, in vitro, cytokines have shown to downregulate NIS, inhibit Tg synthesis, decrease cAMP, decrease TSH-induced TPO mRNA and 5'-deiodinase activity which can all contribute to lower thyroid hormone secretion.

The pituitary production of TSH is radically suppressed considering the low plasma concentrations of thyroid hormone. Not only the thyroid hormones, also the other anterior pituitary hormones IGF-1, LH, FSH (and insulin) are lowered during severe illness. Various theories exist about the pituitary unresponsiveness. The fact that also other pituitary hormones are lowered indicates a role of the pituitary in NTI. The
fact, however, that the impaired pulsatile secretion of GH, TSH and gonadotropin can be re-amplified by relevant combinations of releasing factors indicate that the hypothalamus plays the more important role in prolonged critical illness. Whether to treat NTI is still an issue of debate. Several studies have been performed; some have shown replacement hormone therapy to be disadvantageous, indifferent and in some studies it appears to be beneficial.

In premature infants with severe respiratory distress, prophylactic T₄ and T₃ administration daily was beneficial and lowered mortality. This study was criticized however, due to weakness in methodology and iodine-deficiency in the control patients. Four randomized clinical trials have been published concerning this subject. In all four studies, mortality and morbidity were not significantly improved. Subgroup analyses in one study did show that mortality in T₄ treated infants that had not received antenatal steroid therapy was significantly lower and that T₄ treated infants < 27 weeks gestational age had a better developmental score after 2 and 5 years than controls, and infants born < 29 weeks had a better motor outcome, however, it was also found that the control group of infants born < 29 weeks showed a better cognitive and neuromotor development. In conclusion, it is advised that preterm infants should not be given T₄ treatment.

Children treated with T₃ postoperatively after cardiac surgery, required less cardiac support. The fact that a decreased peripheral conversion of T₄ to T₃ is characteristic for NTI may explain why T₄ administration fails to demonstrate clinical benefit. Considering the hypothalamic role in NTI, the administration of TRH alone or in combination with GH-secretagogues has been suggested but is still under investigation.

It has been recommended that, for adults, therapy should be initiated if serum T₄ levels are depressed below the critical level of 51.5 nmol/l. with T₃ and T₄ supplementation. However, this replacement therapy must still be considered experimental.

For children, the consequences of intermittent episodes of lowered thyroid hormones may be different than for adults because low concentrations of thyroid hormone may not only have a direct influence or be an indicator on outcome of the disease, but may also have effect on other, more long-term parameters, such as growth and (brain) development into adolescence. This has not been investigated.
It can be expected that in children with cancer the concentrations of thyroid function parameters are frequently affected. At time of diagnostics, depending on the localization and stage of the tumor and the clinical condition of the child, NTI may be present. During therapy for the malignant disease, NTI may frequently occur depending on the frequency, type and intensity of the cytotoxic treatment, and during episodes of fever in aplasia, admission to the ICU or following bone marrow transplantation. As these children will encounter such episodes frequently, it can be expected that these hormonal changes are of clinical relevance and may contribute to retardation in growth and development and to changes in metabolic parameters.

In figure 8, the factors that might influence the thyroid function or its determinants during treatment in pediatric cancer patients are illustrated. All factors may influence the thyroid function determinants directly or indirectly by interaction with each other (for example chemotherapy may directly lower TBG or may lead to anorexia which may lead to NTI). Not many studies have been performed on the changes in the thyroid function parameters during treatment in children with cancer.

In children with leukemia and aplastic anemia the thyroid function was measured before and three months after allogeneous bone marrow transplantation. In 48%
abnormal thyroid hormone parameters were found, of which 5\% had a compensated hypothyroidism and 43\% a state of non-thyroidal illness. In this study, 2 risk factors were identified for developing NTI; the administered steroid dose and an age above 16 years. The survival of children with thyroid hormone concentrations within the normal range was 83\% compared to 49\% of children with NTI. From these results it was concluded that NTI is an indicator of a bad prognosis. In 25 children with lymphomas and leukemia, a decrease in plasma T₃ was observed after treatment with chemotherapy. In 57 patients receiving BMT, 42\% were diagnosed with NTI in the first 6 months following transplantation, of which 15.8\% still had NTI after 1 year. Of 7 children with Hodgkin's disease, 5 presented with NTI that disappeared within 6 months. In this study, having NTI was not related to worse outcome in terms of survival or development of thyroid disease. In chapter 9, a prospective evaluation of the changes in thyroid hormone state in children receiving chemotherapy is given.

In summary, in this general introduction it has been demonstrated that in children with cancer, the thyroid function and/or structure may be influenced by the tumor, the treatment or the condition of the child. Damage may already be present during treatment or only become manifest many years after achieving complete remission. Also many effects on other endocrine organs must be expected and anticipated in children treated for a childhood malignancy. These facts indicate the need for detection strategies and ways of prevention, which will be the subject of this thesis, concentrated on the thyroid gland.
1.7: Outline of the thesis

A childhood malignancy and/or its treatment may have numerous effects on the thyroid gland or its function. In this thesis, the occurrence, the consequences and the prevention of thyroid damage during and after treatment for pediatric cancer is evaluated, aiming to develop new guidelines for the detection and prevention of these problems.

Part 1

In this part, thyroid damage which can occur after treatment with radio-labeled MIBG in children with NB is evaluated. In chapter 2, the occurrence of thyroid dysfunction is evaluated in a retrospective cohort of children treated with $^{131}$I-MIBG. In this cohort, potassium iodide (KI) was given to protect the thyroid gland from uptake of and irradiation damage due to $^{131}$I. In chapter 3, a more thorough (long term) follow-up in a group of survivors of NB is performed, cross-sectionally, to evaluate the state of the thyroid function and its consequences after a longer period of follow-up. As these children are given multi-modality treatment, also the other endocrine functions are evaluated. Because we detected a high percentage of thyroid dysfunction in the children with NB after treatment with $^{131}$I-MIBG with KI administration for thyroid protection, a new thyroid protection procedure was developed. In chapter 4, the results of a prospective cohort of children with NB using this new procedure is described. The new thyroid protection procedure consists of the combination of KI, methimazole, and $T_4$ (the dilute-, block- and replace protection).

Part 2

This part of the thesis concerns thyroid damage induced by external (X)-irradiation. To search for ways of prevention of X-radiation induced thyroid damage, a preclinical in-vivo model was needed. In chapter 5, a rat model is described in which we correlate plasma TSH to thyroid histology after various doses of X-irradiation. In chapter 6, this radiation model is used to study the prevention of thyroid damage due to X-irradiation. Several different endocrine interventions are administered during exposure to radiation, aiming to lower thyroid cell activity and hereby attempting to prevent radiation damage.
Part 3
In this part, the adverse events after having a thyroid carcinoma during childhood are evaluated and discussed. In chapter 7, the late effects of treatment for differentiated thyroid carcinoma during childhood are evaluated in a cohort of 25 adolescents. In chapter 8 a case report is presented which illustrates the many difficulties one may encounter during diagnostics and treatment of children with the MEN 2A syndrome, despite early thyroid surgery.

Part 4
The influence of treatment with chemotherapy on the concentration of iodothyronines in the blood circulation during treatment is demonstrated and discussed in chapter 9. Although permanent thyroid damage has been extensively described in literature after exposure to irradiation, the exact additional role of chemotherapy in patients treated with both treatment modalities is still unclear. Chapter 10 describes the damaging effect of chemotherapy adjuvant to damage caused by radiation in a large heterogeneous group of childhood cancer survivors.

Part 5
In this part of the thesis, two systematic searches are presented that summarize all the preventive steps that have been undertaken in literature, including our own of chapter 2, 4 and 6, to prevent the thyroid gland against radiation damage, in humans (11a) and in animals (11b). In chapter 12, the results and hypotheses generated in this thesis are further discussed leading to suggestions for future research and implications for clinical practice.
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