INTRODUCTION

Thyroid nodules affect approximately 20% to 45% of the population during their lifetime, but only a minority of nodular goiters bear a clinically relevant malignant potential. A simple diagnostic approach solving this problem does not exist. Cytological evaluation after fine needle aspiration obtained from thyroid nodules allows only for the detection of thyroid carcinoma in 80% to 90% of the cases. Thus, better methods predicting the malignant potential of thyroid nodules and/or diagnosing existing malignancies are urgently needed. To solve this problem the first step was designating animal models of thyroid carcinogenesis that help to understand this process in more detail. Last century’s investigations in this field showed that the thyroid gland serves as a useful experimental model for understanding tumor formation not only in endocrine systems but, in epithelial tissues in general. Since the mid-1930s, the study of experimental carcinogenesis in rats, mice, hamsters, guinea pigs, sheep and swine has been focus of attention. In vivo, the growth of the follicular epithelium is controlled by a single tropic stimulus, the thyroid-stimulating hormone (TSH), which is secreted by the anterior pituitary gland at a rate dependent on the serum concentration of thyroid hormones (T3 and T4). Inhibition of this feedback loop by reduction or abolition of T3/T4 production leads to an increase in serum TSH. This initially induces an uniform hyperplasia of thyroid follicular epithelium, the first step of tumorigenesis. The discovery of the thyrostatic effect of some naturally occurring substances and the subsequent development of numerous synthetic preparations with the same action
gave an impetus to and provided an opportunity for the systematic investigation of morphological and functional changes in thyroids of experimental animals.

HISTORICAL OVERVIEW
In 1909, McCoy was the first to systematically investigate thyroids of animals. He searched for tumors in 23,000 wild rats, but failed to detect them. Eight years later, Bullock and Rhodenburg identified nine tumors in 4,300 rats. In 1926, Syle et al. found 12 so-called carcinomas in 51,700 mice. All these tumors were spontaneous tumors. After the description of goiter in man (Marine 1924), the era of experiments in the field of thyroid carcinogenesis started sporadically with the investigations of Wegelin (1928), Hellwig (1935), and Hercus & Purves (1936). In the following time, each decade was influenced by leading research groups. In the 1940s, Purves, Bielschowsky, Kennedy and Griesbach (New Zealand, Germany) described the role of low iodine intake and continued the investigation of other positive goitrogenic agents. Kennedy, therefore, synthesized a quantity of allylthiocyanate (mustard oil), from a glucoside in mustard seeds, which were also goitrogenic. This compound was too toxic to be fed to rats, but when treated with ammonia, it was converted to allylthiourea and could be incorporated in the rat diet. It was shown that such drugs are goitrogenic, as is a deficiency of iodine, because they block the production of thyroid hormone. In the 1950s-1970s, Lindsay, Chaikoff (USA) and Doniach (U.K.) described the effects of external and internal irradiation on thyroid tumor production and gave evidence of a dose-dependent relationship. In the 1980s, Hesch, von zur Mühlen (Germany) and Dumont (Belgium) searched for tumor-initiating mechanisms through hormone dysbalances (TSH vs. TRH) and detected morphological changes by electron microscopy. Williams and Wynford-Thomas (U.K.) clarified the functional role of TSH and described differential gene expression (ras) in experimental thyroid tumors for the first time (Lemoine et al 1988). In the 1990s, Brabant, Dralle and Hoang-Vu (Germany) conducting short-term and long-term studies, investigated the regulatory mechanisms of thyroid tumorigenesis and described the changes of histology, ultrastructure, function, and proliferation in detail. In the present time, experiments performed by Japanese and Russian groups (Hirose, Hoshi, Hiasa, Nadolnik) focus on testing the carcinogenic potential of several parts of nutrition and diverse environmental factors.

THE PROBLEM OF TUMOR CLASSIFICATION
All the difficulties encountered in the classification of human thyroid tumors have to be faced in an attempt to classify thyroid tumors in animals, particularly in the rat. In early experiments, the well-known absence of clear-cut histological criteria for distinguishing reactive hyperplasia from neoplasia or benign from malignant tumorous growth has been reflected in publications describing corresponding lesions in the rat. In most cases, the authors have tried to apply the nomenclature of human pathology to the lesions observed in the rat thyroid. This approach has not only some easily recognizable advantages, but also some disadvantages. On the one hand, the use of similar terms would provide an opportunity for conducting a comparative analysis of
Figure 1. Experimental thyroid tumors in rats induced by x-ray irradiation: Low power view of a follicular adenoma with surrounding capsule (a), high power view of papillary carcinoma (b), follicular carcinoma (c) and squamous cell (epidermoid) carcinoma.

Experimental and clinical observations. On the other hand, the same terms might be, and have already been, applied to morphological lesions that are superficially similar, but whose biological behavior is different. The broadest definition applicable to both benign and malignant neoplasms was formulated by Axelrad and Leblond (1955). It fits in with the criteria applied to rat tumors by most experimentalists and can be paraphrased as follows: a pathological change in the rat thyroid can be regarded as a neoplasm when it is focal; it is distinct from the rest of the gland cytologically and architecturally and shows evidence of progressive growth. As to the definition of such terms as “adenoma” and “carcinoma”, the wording suggested here is based on the above definition of a neoplasm and on personal experience gained in studying the peculiarities of behavior of rat thyroid tumors.

An encapsulated epithelial neoplasm without evidence of invasive growth or distant metastasis can be considered an adenoma (Figure 1a). A carcinoma in the rat thyroid is an epithelial neoplasm of any histological structure that shows destructive invasive growth, which leads to metastasis to the body (Figure 1b–d). Apart from clear proof of local invasive growth, there is no convincing evidence of malignant growth except the demonstration of metastases. The tendency to overestimate the malignancy of rat thyroid neoplasms and to diagnose them as carcinomas simply on the grounds of their “malignant” appearance is discernable in many publications. The classification given below, was drawn up under consideration of certain data on embryogenesis of this gland, heterogeneity of its epithelial cell population, and the already mentioned
peculiarities of normal growth of the thyroid epithelium. However, to make this classification more practicable and comparable with that of WHO for human tumors microscopic morphology rather than histogenesis has been selected as a basis:

Benign tumors:
Follicular adenoma (including microfollicular, polymorphofollicular and trabecular adenoma), papillary adenoma, simple solid adenoma, light-cell solid adenoma, and squamous cell (epidermoid) cystadenoma.

Malignant tumors:
Follicular carcinoma (including microfollicular and polymorphofollicular carcinoma), papillary carcinoma, solid carcinoma (including small cell, polymorphous solid and light-cell solid carcinoma), squamous cell carcinoma, sarcomas and mixed tumors (carcinosarcoma).

This classification does not include such entities as leiomyoma, hemangioma, lymphoma, teratoma, neurogenous tumors, and some other neoplasms that have been observed in humans and several animal species other than the rat. An attempt has also been made to avoid the use of proper names that have already led to some diagnostic confusion (for instance Hürte cell tumor or Lindsay tumor). Of the characteristic and predominant histological patterns observed in neoplastic epithelial nodules, the three most common ones (follicular, solid and epidermoid) were selected to designate the main categories of tumors. As mentioned earlier, the proliferation of rat thyroid epithelial cells in solid aggregations must be considered a normal feature. The listed tumors are rarely found in their pure morphological form. Most experimental tumors in animals represent virtually different transitional variations in between these artificially separated entities. The vast majority of follicular neoplasms contain solitary or numerous foci of solid cell nests, and it is the rare solid tumor that does not show areas of follicular structure. An introduction of all the subdivisions covering even the most frequent transitional forms of tumors would make this classification useless. An attempt to classify the endless variety of histological pictures produced by physiological shifts in this correlation is hardly justifiable.

SPONTANEOUS THYROID TUMORS IN ANIMALS
Roe (1965) defined a spontaneous thyroid tumor as a neoplasm that had developed without any influence exerted by internal or external carcinogens. Data on the incidence of spontaneous thyroid tumors in animals are contradictory. McCoy (1909) and Woolley & Wherry (1912) were the first to systematically investigate thyroids of animals. They searched for tumors in 23,000 and 100,000 wild rats, but failed to detect them. In 1917, Bullock and Rhodenburg firstly described nine tumors found in 4,300 rats; in 1926, Slye et al detected 12 so-called carcinomas in 51,700 mice. They are seen more often in laboratory rats, but have a predilection for older animals. Many of these tumors are derived from the C-cell component of the thyroid gland. Lindsay et al (1968) coined the term “naturally occurring carcinoma of the rats thyroid” for these medullary tumors. In the following years, many studies described spontaneous tumors in various rat strains: For example, van Dyke (1944) reported on nine cystadenomas in 16 Wistar rats that died at the age of more than 800 days. According to other reports,
the incidence of spontaneous thyroid neoplasms in Wistar rats is much lower (about 5%; Bielschowsky 1953, van Dyke 1953). In a large group of female Sherman rats kept for more than two years, Axelrad and Leblond (1955) came across only one solid nodule that was composed of light cells. In 1960, Isler et al showed that approximately 40% of female Sherman rats had developed small nodules of light cells at the age of 14 months. In a careful study of serial sections of thyroid glands from Sprague-Dawley rats (mean age 637 days), Thompson & Hunt (1963) observed C-cell tumors in 39% of animals. In contrast, Schardein et al (1968) recorded only 20 follicular adenomas in 5,086 Sprague-Dawley rats. Boorman et al (1972) reported on 123 cases of naturally occurring medullary carcinomas among 334 WAG/Rij rats, 84% of which were older than two years. Lindsay et al (1968) investigated various rat strains and found medullary carcinomas in 19% of Wistar, 22% of Fischer, 22% of Sprague-Dawley, and 40% of Long-Evans rats. Hamsters were also occasionally used for experimental tumorigene-
sis; Pour et al (1976) found spontaneous thyroid adenomas in 5–10% and carcinomas in 1% of untreated Syrian hamsters. Summarizing the data of the above-mentioned studies, one can draw the following conclusions: the vast majority of spontaneous thyroid tumors so far observed in rats maintained under conventional conditions, i.e., not subjected to factors that either continuously increase the output of TSH or have direct carcinogenic effects, is represented by solid neoplasms. These tumors appear as nodules of different sizes, composed of several varieties of large, oval or polygonal pale cells that neither form follicular structures nor produce colloid. Such nodules usually grow slowly, despite the fact that growth is infiltrative in many cases. The incidence of the neoplasms increases with age. Most of them are revealed in two-year-old or even older rats. The origin of spontaneous solid thyroid tumors in rats has been attributed to the intrafollicular light cells (Askanazy/Hürthle-cells) and to the parafollicular C-cells. In normal rats, spontaneous tumors of follicular, adenomatous and papillary pattern have been observed much less frequently than the above mentioned neoplasms, and in these rare cases, they were consisted of colloid cysts or small hyperplastic nodules.

**EXPERIMENTAL INDUCTION OF THYROID TUMORS**

Methods for inducing thyroid tumors in animals can be subdivided into two groups according to the mechanism of action. The first group comprises methods based on the application of substances with a direct oncogenic effect on thyroid cells, i.e., proper carcinogenic agents. The methods in the second group aim primarily at establishing a hormonal imbalance that, in turn, will lead to tumor development. Such a division, however, is rather artificial. Some known carcinogenic agents with a direct mechanism of action may also produce profound and irreversible hormonal disturbances that lead to thyroid carcinogenesis. Vice versa, many of the agents used to disturb the hormonal balance may exert a direct carcinogenic effect.

**Tumor induction by elevation of TSH**

As a result of numerous investigations, a consistent concept of experimental thyroid tumor pathogenesis was established to explain the tumorigenic effect of antithyroid drugs (Bielschowsky 1955). According to this concept, the first stage in the
development of thyroid tumors is inhibition of hormone production by the thyroid tissue under the influence of goitrogens. The second stage is the sustained intensification of synthesis and release of TSH. Continuous excessive secretion of TSH is assumed to be one of the basic pathogenic factors responsible for thyroid tumor development.

Goitrogen-induced tumors

Experimental goitrogen–induced tumorigenesis began with the observation that prolonged feeding of a diet containing plants of the Brassica species produced goiter in rats (Hercus & Purves 1936) leading to a high yield of adenomas (Griesbach et al. 1945). Kennedy (1942) suggested that the active agent in the rape seed was a urea derivative. Numerous investigations have previously revealed that in the thyroid the family of thiourea derivatives is both goitrogenic and carcinogenic. For example, Paschkis et al. (1948), Kuzell et al. (1949), Clausen (1953), Wollman (1961), and Grundman & Seidel (1965) used thiouracil; Doniach (1950), Christov (1968), and Jemec (1977) used methylthiouracil (MTU); Van Dyke (1953) and Sellers & Schonbaum (1957) used propylthiouracil (PTU); Ulland et al. (1972), Graham et al. (1975), and Arnold et al. (1983) used ethylenethiourea (ETU); tetramethylthioura (TMTU) was used by Stula et al. (1979). Although there is some variation in the goitrogenic activity of the different thioureas, subsequent development of tumors appears to be a uniform finding for all members of this family of thiourea derivatives. They all inhibit steps in hormone synthesis (coupling of iodothyrosines, iodination of thyrosines and monoiodothyrosines) and some, such as PTU, also inhibit deiodination. Daily intake of 5–10mg PTU or 10–20mg MTU is considered an optimum dose for tumor development. Another compound with goitrogenic and carcinogenic activity is the herbicide aminotriazole (ATA). Jukes & Shaffer (1960) and Napalkov (1967) found a similarly high tumor incidence (25% adenomas) in rats of both sexes following lifelong 0.01% ATA administration. Tsuda et al. (1976) and Steinhoff et al. (1983) were able to produce carcinomas in Wistar rats. ATA has turned out to be an experimental goitrogen because the level of general toxicity was lower, than that observed in the thiourea group (Gibson & Doniach 1967). In contrast to mice and rats, hamsters appear to be relatively resistant to tumorigenesis caused by goitrogenic agents. Steinhoff et al. (1983) showed that irrespective of the amount of dosage given to hamsters ATA produced no increase in thyroid neoplasia, while PTU produced thyroid hyperplasia but no neoplasia (Kirkman 1972). However, MTU is reported to produce adenomas and carcinomas in hamsters with frequency and latent intervals similar to those in rats and mice (Akimova et al. 1969, Christov & Raichev 1972). In addition, Hellwig & Welch (1963) described the development of thyroid tumors in 15% of guinea pigs after 14 months PTU intake.

Tumor induction by low-iodine intake

Thyroid tumors have been induced in rats by prolonged over-stimulation of the gland with endogenous TSH only. This method involves maintaining the animals in a state of chronic iodine deficiency. The first observations of rats kept in such a state can be traced back to Bircher (1910, 1911). Since then, the method has been perfected by several groups (Hellwig 1935, Bielschowsky 1953, Isler 1959, Al-Saadi 1968 a.o.), but
the most detailed examination was performed by Axelrad & Leblond (1955). These authors found that the changes seen in the thyroid gland and pituitary gland with a low-iodine diet are identical to those seen under long-term goitrogen administration. Thyroid adenomas could be induced in almost all experimental rats when maintaining an iodine-restricted diet with daily intake of about 0.7 μg for two years. However, the malignancy of the follicular neoplasms that arise in the rat thyroid as a result of chronic iodine deficiency is questionable. In all cases reported so far, the frequency of carcinomas originating from follicular epithelium is clearly lower in rats kept on a low-iodine diet than in animals treated with goitrogens. In rats, iodine deficiency is a much more effective tumor promoter than is a carcinogen, suggesting that a similar relationship may exist in human populations (Ward & Oshima 1986). In C3H/Hey strain-mice, Schaller & Stevenson (1966) used low-iodine diet to induce benign and malignant thyroid tumors; after one year of treatment, carcinomas developed in 14%. In hamsters, Fortner et al. (1959) found well-differentiated, metastatic follicular tumors in 18% of females, but no lesions in males after a 70-week iodine-deficient diet.

**Tumor growth after partial thyroidectomy**

Although it has been claimed that subtotal thyroidectomy is a potent method that raises the level of trophic stimulus to the thyroid, the yield of tumors in animals treated in this way is lower than in animals given a low-iodine diet or long-term goitrogen (Domach 1970). Doniach & Williams (1962) and Goldberg et al. (1964) induced 14% adenomas and 4% carcinomas in Lister rats 15 months after 85%-excision of thyroid mass. In contrast, Ird (1968) found that subtotal thyroidectomy alone did not increase the incidence of tumors above that seen in control rats, and that surgery reduced the incidence of tumors in rats treated with MTU (25% vs. 74%). The relatively low yield of tumors obtained by this method may be explained by the fact that owing to surgery most part of the target gland responsible for the trophic stimulus is removed. This, of course, reduces the population of cells that might undergo neoplastic change.

**Tumor induction by ionizing radiation**

Radiation affecting the thyroid gland is possible via two routes: external or internal. External administration of radiation is achieved by using either X- or gamma-emitting radiation sources. Theoretically, this route has the advantage of permitting delivery of a precisely calculable amount of rads (Gray), but suffers from several practical problems. Firstly, given the size of the thyroid of the rat or mice, it is difficult both to localize the target and to avoid damaging the surrounding tissue. Secondly, it is also essential, but difficult, to avoid unirradiated parts of the thyroid, leading to an overestimation of the dose delivered. These problems may be solved by lightly anaesthetizing animals and by carefully placing a lead collar with a small window over the neck region, both to protect the surrounding tissue and to hold the animal in position. Internal irradiation is usually given in form of sodium salts of I\(^{131}\) or I\(^{125}\) by intraperitoneal or intrathyroidal injection. This method is much more convenient than external irradiation, although different problems arise. Accurate calculation of the dose received is difficult, since it is dependent both on percentage uptake and retention of the isotope. The amount of
isotope retained by the gland is dependent on the biological, as well as on the physical, half-life. The biological half-life is difficult to predict and may vary with the strain of animal species, diet and sex. Even the surrounding temperature has been shown to influence iodine uptake (Doniach 1950).

Tumor growth following internal radio-iodine application

The destructive effect of variable amounts of radioactive iodine on the normal thyroid was shown for the first time in 1942 by Hamilton & Lawrence in the dog and rabbit and in 1948 by Findlay & Leblond in the adult rat. Experimentally induced neoplasms of the rat thyroid following $^{131}$I-irradiation were first produced by Doniach (1950). He reported that the administration of $32\mu$Ci $^{131}$I significantly increased the formation of adenomas, as compared with untreated groups. In 1951, Goldberg & Chaikoff showed that a single dose of $^{131}$I would cause benign and malignant tumors after a period of 1.5–2 years. Since these initial experiments, several pieces of evidence have shown that as little as $5\mu$Ci $^{131}$I is tumorigenic to the rat thyroid, and that the administration of doses ranging from $5–400\mu$Ci $^{131}$I can produce benign and malignant neoplasms. However, with increasing dose, a larger percentage of cells is sterilized, thus reducing the number of tumors produced (Table 1). The optimal carcinogenic radiation dose to young adult rats is approximately $5–50\mu$Ci $^{131}$I. One important factor affecting the dose-response curve for tumor production is the age at which radiation is given. Doniach (1969) showed that after administration of $2.9\mu$Ci $^{131}$I at birth tumor yield was similar to that following $30\mu$Ci $^{131}$I given to adult rats. Taking into account the weight

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<th>Dosage ($\mu$Ci $^{131}$I)</th>
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of the animals, his results do not reveal any increased susceptibility of the newborn rat to the carcinogenic action of radioactive iodine compared with adults. There is also no marked difference in weanling rats (28 days) as compared with young adults (70 days) (Doniach 1957).

**Tumor induction after external X-ray irradiation**

Experimentally, the effects of external irradiation on thyroid tumor production have mostly been assessed in rats. In several studies, doses ranged from 1 Gy to 20 Gy, including various intermediate doses. Table 2 shows that the optimum dose for the development of tumors is dependent on the age at which the radiation is initially administered and, obviously, the age at which the animal is examined. For 3-month-old rats, the optimal dose for thyroid tumor induction using only X-rays lies between 5 and 10 Gy. A lower dose of X-rays given to the neck region of 10-day-old rats has induced more tumors with a shorter latent period than the same dose given to 60-day-old animals (Christov 1978). This increase in tumorigenicity in younger animals is possibly due to the higher mitotic index of the thyroid in younger rats.

Comparisons of X-ray and $^{131}$I doses, which are able to produce thyroid tumors have shown a ratio of 1:8 to 1:10 (Doniach 1956, 1963). Abbatt et al. (1957) investigated the inhibition of goitrogenesis in rats, produced by varying doses of X-rays and radioactive iodine. After giving either 30$\mu$Ci $^{131}$I or 10 Gy of X-rays their results showed a similar effect. They suggested that compared with $^{131}$I the apparent ten-fold increased sensitivity of the thyroid to X-rays as may be due to the overall uneven distribution of radiation with $^{131}$I so that some follicles possibly receive a smaller amount than those absorbed by others.

**Thyroid tumorigenesis induced by chemical carcinogens**

In 1942, Esmarch was the first to use chemical carcinogens for the production of thyroid tumors in experiments. He applied methylcholanthrene directly to thyroid glands of rats. The direct application of other carcinogenic polycyclic hydrocarbons

### Table 2. Review of the literature: incidence of thyroid tumors in rats following a single bilateral irradiation with X-rays

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<tr>
<th>Dosage (Gy)</th>
<th>Age (days)</th>
<th>Latent period (month)</th>
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was studied later and, as expected, such an approach produced more sarcomas and squamous cell carcinomas than adenocarcinomas. Money & Rawson (1950, 1965) found that administration of dimethylbenzanthracene either directly to the thyroid gland or by subcutaneous injection did not significantly alter the incidence or type of produced tumors.

Tumor induction by aromatic amines and azo dyes

Thyroid adenomas can be produced by systemic administration of acetylaminofluorene (AAF, formerly used as an insecticide), which can also produce a high yield of tumors in other organs of rats, including mammary gland, liver, kidney, intestine and uterus (Bielschowsky 1944). Murthy (1980) found that continuous administration of the dye intermediate, 4,4’-methylene-bis-(N,N-dimethyl)-benzenamine (MDBA), to F344 rats of both sexes produced follicular tumors after 80 weeks. The incidence was higher in animals receiving larger doses, and it was in these animals only that hyperplastic changes were seen in the thyroid. Murthy et al. (1985), taking up earlier studies of 4,4’-oxydianiline (ODA) that had shown evidence of a goitrogenic and carcinogenic effect on the thyroid in rodents (Hayden et al. 1978), in whom it also caused a high incidence of liver tumors. They reported that the incidence of thyroid hyperplasia and neoplasia was high in animals given 0.04%–0.05% ODA in the diet. The earliest follicular tumor was seen at 28 weeks, and after two years, neoplasms were present in 86% of the survivors of these groups. Thyroid hyperplasia and changes in thyrotroph population in the pituitary glands of rats with follicular tumors suggested that the carcinogenic effect of ODA was at least partly mediated through an elevation of TSH.

Tumor growth induced by nitrosamines

Diisopropanolnitrosamine (DIPN) is one member of a family that belongs to the carcinogenic nitrosamines, i.e., postulated intermediates of the parent compound di-n-propylnitrosamine, with broad-spectrum activity in most species tested. When given weekly subcutaneous injection to Sprague-Dawley rats for life, there was up to 50% incidence of thyroid tumors, with short latent intervals of 23 weeks for adenomas and 26 weeks for carcinomas (Mohr et al. 1977). N-bis-(2-hydroxypropyl)nitrosamine (DHPN) can also initiate thyroid tumors, although the frequency is lower in the absence of a promoting agent. Obviously, there is a difference in the sensitivity of rat strains to this agent: equitoxic doses resulted in thyroid tumors in 50% of Sprague-Dawley rats and in 20% of MRC rats (Mohr et al. 1977, Pour et al. 1979). N-nitrosobis (2-oxopropyl)amine (BOP) is a nitrosamine closely related to DHPN, with a high yield of thyroid tumors in rats: Pour & Salmasizadeh (1978) found 50% incidence in MRC rats following a single dose, and 60% after weekly treatment for life. When given in equitoxic doses, BOP produced thyroid neoplasms in 60% and DHPN in 20% of MRC rats (Pour et al. 1979). In 1986, Pour also showed that intrauterine exposure of hamsters to BOP results in 50% incidence of thyroid adenomas in female animals, but 0% in male animals; this is of particular interest as in adult hamsters, the carcinogenic effect is almost entirely confined to the pancreas.
Thyroid carcinogenesis with N-nitroso-N-methylurea (NMU) was first described by Jobst (1967). He treated rats during their intrauterine development and after birth with weekly injections and found thyroid carcinomas in some of the survivors. NMU (3 injections of 30mg/kg) was given to Wistar rats by Thomas & Bollman (1974), who reported a 100% incidence of follicular cell carcinomas after 7–8 months. Takizawa & Nishihara (1971) reported the induction of a thyroid carcinoma in a female rat given the powerful neurocarcinogen N-nitrosobutylurea (NBU). N-ethyl-N-nitrosourea (ENU) has been extensively applied as a transplacental neurocarcinogen in several species, but the use of high doses in young rats can produce thyroid tumors, mostly of encapsulated papillary type, in a small portion (Stoica & Koestner 1984). Warzok et al. (1977) and Napalkov et al. (1981) reported that transplacental administration of ENU to dogs causes not only early development of thyroid tumors in a minority of cases, but also to late tumors.

Studies with combination of tumor-inducing factors

The methods of thyroid tumor induction outlined above, i.e., treatment with antithyroid or carcinogetic substances, restriction of iodine consumption and irradiation, are often used in various combinations. For instance, pre-irradiation of the thyroid significantly increased the tumor development in rats kept on a low iodine diet (Nadler et al. 1969). Similar effects have been observed in experiments with the combining irradiation with antithyroid drugs or certain chemical carcinogens (Lindsay 1969). The combination of antithyroid drugs or a low-iodine diet with chemical carcinogens (AAF was usually used) also resulted in accelerated tumor development (Bielschowsky 1944, Hall & Bielschowsky 1949, Axelrad & Leblond 1955). Hall (1948) made the interesting observation that even relatively small doses of AAF given for a period as short as 1 week were sufficient to produce an enhancing effect on tumorigenesis, and this effect was still observed when goitrogen treatment with allylthiourea was delayed for up to 18 weeks. There is evidence that AAF causes neoplastic progression of thyroid epithelium only under conditions of excess TSH stimulation. Otherwise no detectable effect was observed by Bielschowsky & Griesbach (1950) a finding not confirmed by other studies (Grundmann & Seidel 1965). It is possible that owing to a toxic alteration of hepatic cells normally responsible for hormone degradation AAF itself might indirectly raise TSH levels. Hiasa et al. (1982) found that treatment of rats with either repeated small doses of DHPN or treatment with aminotriazole (ATA) did not produce tumors, but combination therapy with both agents could yield neoplasms in up to 100% of animals after 12 weeks. PTU has also been used successfully in combination with DHPN by this group (Kitahori et al. 1984). Several studies have given evidence of the promoting effect of barbiturates on thyroid carcinogenesis by DHPN. Whereas single or multiple administrations of DHPN alone resulted in 0% tumors at week 20 in rats, additional treatment with barbital for 12 weeks produced thyroid neoplasms in 45% of animals; phenobarbital treatment was even more effective, leading to an incidence of up to 100% of animals at week 20 (Hiasa et al. 1983). Schaffer & Müller (1980) found...
invasive thyroid tumors by 16 weeks and pulmonary metastases by 30 weeks after 3-times NMU injection in combination with long-term MTU treatment. Similar results were obtained by combining NMU with PTU in F344 rats (Milmore et al. 1982), and NMU with phenobarbital (Tsuda et al. 1983). Ohshima & Ward (1984) found that iodine-deficient diet was also an efficient promoter for NMU-initiated tumors, with 100% incidence of thyroid tumors in F344 rats after 20-week treatment. Even without the additional influence if iodine-deficient diet, NMU treatment resulted in 10% incidence of adenomas after 20 weeks, 70% incidence after 33 weeks, and 10% incidence of carcinomas after 33 weeks. Diwan et al. (1985) used NMU in combination with barbiturates and demonstrated that the incidence and multiplicity of thyroid tumors are greater in male rats, because in this sex phenobarbital seems to be a more effective promoter, as shown by the higher incidence of liver tumors in male animals in the same experiment.

Long term study for investigating changes in morphology, function and proliferation of thyrocytes after varying nutrition iodine and external radiation in rats

Several studies mentioned above have addressed the question of a predominant iodide-dependent regulation in the development of hyperplasia and proliferation of thyroid follicle cells in vivo, or have reported a major contribution of TSH in this respect. However, most of these studies, based on severe iodide depletion, used a short observation period for defining iodide-dependent effects. Only few reports have dealt with the ontogenesis of morphological and hormonal changes during moderate long-term iodine deficiency, which more closely parallels the situation of humans in an iodine-deficient area. The long-term effects of iodine excess in humans have not been studied in detail, but recent reports suggest that iodine excess also induces goitrogenesis and benign thyroid tumors.

One study (Boltze et al. 2002) aimed at systematically monitoring the influence of a long-term increase or decrease in daily iodine supply on the morphology of the thyroid of rats. To develop a reproducible model of thyroid tumorigenesis, this treatment was combined with short-term external radiation of the thyroid using known environmental hazards. It is expected that such a model helps to define the relevant genetic alterations causing thyroid tumor formation in a subsequent step, and may thus contribute to the diagnosis of thyroid adenomas.

Experimental design

Three groups of 80 male Sprague-Dawley rats (28d old), each differing in daily iodine supply, were investigated: 1. normal iodine intake (7000ng Iodine/100g body weight/day)(In), 2. low iodine diet (420ng Iodine/100g bw/d (I-) and 3. high iodine diet (72000ng Iodine/100g bw/d. On day 40, the thyroid region of half of each group was externally irradiated with a single dose of 4Gy X-rays (InR, I-R, I+R). Weekly, the animals were monitored as for their body weight, and blood samples for determining TSH, T3 and T4 were obtained. Of each of the six groups, 10 animals were killed at weeks 15, 35, 55 and 110; the thyroids were removed and investigated by histology.
and immunohistochemistry. The following parameters were detected: follicles/mm², colloid diameter, index of fibrosis, proliferation rate and number of tumors.

**Results**

Iodine-dependent changes without radiation:

Iodine deficiency led to lower daily growth rates and a significantly lower final mean body weight of 430g (I-) vs. 501g (In) vs. 475g (I+). The growth process was finished after 18 weeks in I+ and after 21 weeks in In and I-. Long-term iodine deficiency significantly decreased plasma T3 and T4 concentrations after week 9. In contrast, the high iodine diet did not change thyroid function. All changes manifested themselves in alterations in thyroid morphology. Iodine deficiency was associated with significantly large, but fewer, follicles, whereas the high iodine diet led to a significant decrease in the diameter, but to an increase in the number of follicles. The mitotic activity of thyrocytes was very low under normal iodine intake conditions (<1 ±0.2%). Not only iodine deficiency, but also higher iodine intake significantly increased proliferation.
Figure 2. Changes of T3, T4 and TSH plasma concentrations in rats without (A) and after radiation with 4 Gy (B) under normal nutrition iodine conditions (white circle (○)), iodine deficiency (black circles (●)) and iodine supplementation (black triangles (▲)).
Figure 3. Histological data of the thyroids after 15, 35, 55 and 110 weeks under normal iodine conditions (In; black bars ), iodine deficiency (I-; gray bars ) and iodine supplement (I+; dark-grey bars ) with or without irradiation (n = 10; mean±SEM).
(* = statistical significance In vs. I- or I+, p < 0.05; # = statistical significance I+ vs. I-, p < 0.05)
rates. At week 55 and 110, all non-irradiated animal groups were free of malignant
tumors, and benign tumors were not detected until week 55.

_Iodine-dependent changes after radiation:_

After radiation, there were increases in T3 and T4, and a significant decrease in TSH
in the group with iodine deficiency. The hormone concentrations of the normal iodine
and high iodine groups were not significantly altered. In all groups, thyroid weight was
not significantly influenced by radiation. Histologically, the most important finding
in irradiated low iodine diet thyroids was the total destruction of follicles observed at
week 15. After this destruction, a short-term increase in T3 (7th week) was measured.
After the 55th week, a complete restitution of the follicle structure was seen. In contrast
to the sole manipulation of iodine intake, radiation treatment led to a higher number
of benign tumors, starting 55 weeks after having changed nutritional iodine supply,
and to malignant tumors after 110 weeks. Parathyroid carcinomas were also induced:
squamous cell carcinomas of the cervical soft tissue and adenocarcinomas of the salivary
glands. The thyroid carcinomas were solitary tumors, their size ranged between 0.1
and 1.5mm. Neither local lymph node metastasis nor distant metastasis was found.

**Conclusions**

This animal model clearly supports the concept that in thyroid carcinogenesis, there
is a very long latency period between the mutational event and the development
of malignant changes. This contradicts previous studies using a higher stimulation of thyrocyte proliferation by iodine deficiency, where malignancies were detected after much shorter time intervals (Axelrad & Leblond 1955). Large doses of iodine may induce thyroid carcinomas (Correa & Welsh 1960). We showed that mild iodine excess is not necessarily associated with the formation of thyroid malignant neoplasms, but when combined with a mutagen, carcinomas arise with high frequency. These data on mild forms of high iodine intake thus put a note of caution to a long term-use of high iodine. It was shown that euthyreosis is best protection against thyroid cancer before environmental hazards are effective.

The well-defined setting in these experiments clearly demonstrates that mutational lesions acquired by radiation are clinically silent over a long period of time. It is tempting to use such a model to search for candidate genes altered by mutagens, but which are not changed in thyroid adenomas found under control conditions. The definition of such changes may then have important implications for the characterization of the malignant potential of a given adenoma well before cytological or histological changes occur.

FUTURE OF ANIMAL MODELS INVESTIGATING THYROID CARCINOGENESIS

In the last 75 years, very different models investigating thyroid carcinogenesis have been developed. The concept of initiation and promotion of carcinogenesis is well demonstrated by the studies reviewed in this chapter. The initiation step may be produced by diverse agents, including ionizing radiation and many classes of carcinogens. The action of these agents is promoted by raising the level of trophic stimulation (TSH), which can also be achieved in a variety of ways (for instance goitrogen therapy or low-iodine diet). All these models successfully described the changes in morphology and function of thyrocytes during carcinogenesis. However, the time in which these kind of models were used is over. Therefore, in the last ten years, such studies were published only sporadically. The molecular basis of thyroid neoplastic processes involved in experimental tumors is now being elucidated by investigations of the changes associated with developing tumors, and also by the reconstruction of the tumor phenotype through the introduction of genes into thyroid cells. In the last few years, many new methods (including chip- and array technology, or proteomics) have been developed to clarify tumor-related mechanisms and to search for tumor-associated genes. The tumor induction models mentioned above can serve as the basis for yielding material of different stages of thyroid carcinogenesis. A factor limiting these forthcoming gene expression studies is the small amount of relevant material. The use of more sensitive methods will solve this problem in future.

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