A STUDY ON THE INDUCTION OF THYROID TUMORS IN
RATS USING X-IRRADIATION IN CONJUNCTION WITH A
GOITROGEN

by

Peter Mahler

MASTER

Submitted in Partial Fulfillment
of the
Requirements for the Degree
DOCTOR OF PHILOSOPHY

Supervised by
Dr. George W. Casarett

Department of Radiation Biology
and Biophysics
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197⁹₈

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VITAE

Peter A. Mahler was born [REDACTED]. He received the B.S. degree in physics and mathematics from Valparaiso University in 1970, and the M.S. degree in physics from the University of Illinois in 1971. After teaching and working in physics for two years, he entered the University of Rochester in 1973 as a graduate student in the Department of Radiation Biology and Biophysics. His research at Rochester was supervised by Dr. George W. Casarett.

ACKNOWLEDGEMENTS

Working under Dr. George W. Casarett has been the most significant event in my scientific life. His knowledge of the biological, chemical, and physical principles relevant to radiation biology is outstanding. More significant than this is his ability to analyze a problem, to pose an appropriate question, to detect flaws in an argument. One of my favorite phrases in his repertoire generally came when I was attempting to interpret some experimental results, "Turn the coin over and look at it from the other side. Examine it from all possible angles. See how much information you can get out of it." Advice which, I trust, I will always remember. I am proud to have been a student in his laboratory.

The training of a physical scientist in the ways of the life sciences can be a long and arduous task. Part of the credit for making a biologist out of me belongs to Ovide Corriveau, Rick Harding, Al Thomson, and Harry Welch. They spent many hours in the lab with me, talking not only about hockey and vacations, but also about histological techniques and animal handling methods. I thank them for their guidance and their friendship.

A similar vote of thanks for knowledge and friendship goes to Drs. Marty Rosenblum, Dan Rappaport, and Mike King, my predecessors and colleagues in the lab.

A special thank you belongs to Marilyn Campbell, Administrative Assistant Extraordinaire and good friend, who made the stay in

Rochester much more enjoyable for Annette and me. Successfully completing the maze that is graduate education would have been nigh impossible without Marilyn's assistance.

To the members of my committee: Dr. David Weber, who took the time to share views on the physical aspects of Nuclear Medicine and the RPO, and who allowed me to use his gamma counter; Dr. Solomon Michaelson, who always had time to answer a question, and who gave me insights about microwaves, international science and standard setting; and Dr. Louis Hempelmann, who took time from his busy schedule to join my committee, I owe a strong and deep felt thank you. Thanks also go to various members of the biostatistics department, who helped me in the statistical analysis of my data.

I want to thank Bev Holloway for typing this thesis. I appreciate the fact that she always had a smile and cheerful word for me, no matter how poor my handwriting.

To Archie Smiley and Naaman Squires who took care of my rats: Thanks for the good animal treatment, and for the conversation, even though I was a student.

I would also like to acknowledge the role Dr. J. N. Stannard played in my education. Although he did not participate per se in the research that I did, he was a very positive influence on me in my training. He furnished education, advice when asked, and a character and integrity worthy of emulation. I am honored to call him a friend.

Most importantly, I want to acknowledge the assistance of my wife Annette. Without her support, encouragement, prodding and sharing, this work would not have been accomplished.

ABSTRACT

This research was designed to examine the influence of acute localized thyroid X-irradiation and chronic goitrogen administration, separately or combined, on thyroid tumor formation in mature female Rochester ex-Wistar rats.

In the first experiment, the radiation doses were 0; 80; 160; 320; or 640 rads, and the dosages of goitrogen were 0, 4, or 40 parts per million (ppm) of 1 methyl - 2 mercaptoimidazole (methimazole, or MMI) in the drinking water. From the third month, serum thyroxine (T_4) levels were determined monthly. The surviving rats were sacrificed 13 months after the start of the experiment. The thyroids were examined and classified on the basis of 1) presence or absence of tumor, 2) tumor type, and 3) tumor extent. Non-tumorous thyroid tissue was quantified in terms of relative areas of histologic sections occupied by 1) vasculature, 2) follicular epithelium, 3) colloid, and 4) connective tissue.

As evaluated by the χ^2 test statistic, the incidence of rats with thyroid tumors in any treated group receiving 0 or 4 ppm MMI (0 to 30%) was not significantly greater than the incidence in the nontreated control group (6%). However, the incidence in any of the 40 ppm MMI groups (65-100%) was significantly greater than that in the nontreated control group. At all the radiation doses other than 80 rads, the incidence was significantly greater than that in the non-irradiated group (χ^2 , $p < .05$). The 87% incidence in the 80 rad

group was not significantly greater statistically than the 65% incidence in the non-irradiated group. The χ^2 test did not reveal any statistically significant difference in the incidence of rats with thyroid tumors on the basis of radiation dose size, although an increase in incidence with increasing radiation dose was seen in the 40 ppm MMI group. The incidence was so high at the low dose of 80 rads (87%) that there was little margin for further increase with further increase in radiation dose.

The incidence of naturally occurring thyroid medullary tumors did not change with any of the treatment used. Except for one rat, at 640 rads and no MMI, all twelve rats with thyroid carcinomas were in the 40 ppm MMI groups and the incidence appeared to increase with increasing radiation dose (5% at 0 rads to 20% at 640 rads) but not significantly.

Quantitative histology revealed significant MMI dosage dependent increase in mean relative area of follicular epithelium and decrease in mean relative area of colloid. The mean relative area of connective tissue increased with increasing radiation dose.

The mean serum thyroxine levels at 40 ppm MMI, 4 ppm MMI, and 0 ppm MMI were 1.9, 3.5, and 3.7 $\mu\text{g}/100\text{ ml}$, respectively. No marked effect of thyroid irradiation on mean serum thyroxine levels was seen.

In the second experiment, rats receiving 200 ppm MMI in the drinking water and localized thyroid irradiation were sacrificed at 7-1/2 months after onset of treatment. Nearly all (93%) rats in the 0 and 80 rad groups and all in the 160, the 320, and the 640 rad groups

had thyroid tumors. As in Experiment I, thyroid carcinoma incidence tended to increase with increasing radiation dose.

In the third experiment, serum T_4 levels were measured for 12 weeks in rats receiving methimazole or thyroid irradiation, or both treatments. Rats receiving 640 rads + 0 ppm MMI showed a slight decrease in serum T_4 as compared to nontreated controls, while no change in serum T_4 levels was seen in rats receiving 0 rads + 4 ppm MMI or 640 rads + 4 ppm MMI. All rats receiving 40 ppm MMI, regardless of radiation dose, showed decreased serum T_4 levels by one to three weeks after onset of treatment.

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I. BACKGROUND

A. The Rat Thyroid

1. Histology

In the rat, as in man, the thyroid is a bilobed gland, somewhat resembling a butterfly or horizontal figure 8. The thyroid lies in the cervical region, where it is attached to the trachea, just below the larynx. The two lobes lie on the lateral sides of the trachea and are connected to each other by a narrow isthmus of thyroid tissue. The two lobes are not symmetrical with respect to the isthmus; rather, two thirds of the gland lies above the isthmus, and one third lies below. The basic shape of the glands is ellipsoidal, although the top of the lobe is generally wider than the bottom. This shape gave rise to the German name of the thyroid gland: Schilddrüse or Shield Gland. The typical dimensions of a thyroid gland in the rat depend on the size of the animal. For a 275 gm Rochester ex-Wistar female rat, dimensions on the order of 5 mm x 3 mm x 2 mm for each lobe are typical. The weight of the thyroid in the mature male or female rat, as given by McCoy (1952), is 7.5 mg/100 gm body weight. Lu (1976) measured a value of 4.2 mg/100 gm in one-year-old male Long-Evans rats.

The thyroid gland is composed of spherical cyst-like follicles lined with a simple epithelium and containing a gelatinous colloid (Bloom and Fawcett, 1975). Lu (1976) studied the change in follicle size with age in male Long-Evans rats. He reported that some

variation in follicle size with age did occur, but that the general pattern of smaller central follicles and larger peripheral follicles was present in animals 8 to 60 weeks old.

The follicles of the thyroid are surrounded by a rich vascular bed, which facilitates both the uptake of iodine and the release of thyroid hormone by the follicular epithelial cells. In addition to the epithelial cells which make up the follicles, the thyroid contains a second type of parenchymal cell, the parafollicular cell (light cell, clear cell, or C-cell) (Bloom and Fawcett, 1975). These cells are primarily located between follicles, although an occasional C-cell will appear to be part of a follicle. However, close inspection generally reveals that the end of such a cell is not in contact with the colloid of the follicle. C-cells are generally ascribed the function of calcitonin synthesis and secretion (Ingbar and Woeber, 1974). In 1968, Lindsay and co-workers proposed that naturally occurring tumors of the rat thyroid arise from C-cells, a conclusion based in part on the observation by Axelrad and Leblond (1955) who reported that what they referred to as gamma (γ)-nodules in the rat thyroid gland were composed of cells which appeared to be similar to thyroid light cells.

2. Physiology

a. Thyroid hormone level regulatory mechanisms

The primary functions of the thyroid gland are the synthesis, storage, and secretion of the thyroid hormones tri-iodothyronine (T_3) and tetra-iodothyronine (thyroxine, or T_4). The regulatory mechanisms

for these functions are multiple, complex, and not completely understood.

At a minimum, regulation of thyroid function involves thyroid stimulating hormone (TSH or thyrotropin), T_3 , T_4 , thyrotropin releasing hormone (TRH), thyroid iodine levels, and in certain cases long acting thyroid stimulator (LATS) (Ingbar and Woeber, 1974). LATS is not regarded as an important thyroid regulator, except in certain disease states. Current evidence suggests that LATS is a plasma protein of the IgG fraction, and that it acts, as does TSH, by binding to some part of the follicular epithelial cell (Mehdi et al., 1977).

Figure 1 is a diagrammatic representation of the major control mechanisms for regulating thyroid function, as put forth in the review by Ingbar and Woeber (1974). As outlined by Dumont (1971) and Tong (1974), an increased level of TSH causes many changes in thyroid metabolism, the net result of which is follicular cell hypertrophy and hyperplasia, and increased thyroid hormone synthesis and secretion. TSH secretion, as stated by Garcia and co-workers (1976), "...is governed by two main interacting forces. A hypothalamic hormone, identified as the tripeptideamide (pyro) glu-his-pro-NH₂ (TRH), stimulates secretion, and probably synthesis, of TSH. The thyroid hormones L-thyroxine (T_4) and tri-iodo-L-thyronine (T_3) counteract this stimulatory effect of TRH by mechanisms which have not yet been clearly defined and are still mostly speculative. There is convincing evidence that both TRH and the thyroid hormones exert

FIGURE 1

Regulatory Mechanism for Thyroid Hormone Secretion

their effects directly at the pituitary level, presumably on the thyrotrophs." TRH is synthesized in the hypothalamus and transported to the pituitary. The route of transport is generally regarded as being the hypothalamic-hypophyseal portal circulation (Ingbar and Woeber, 1974).

Thyroid synthesis and secretory activity is also affected by thyroid iodine levels. Ingbar and Woeber (1974) stated that the thyroid gland exerts autoregulation, the purpose of which is "to maintain constancy of thyroid hormone stores". The mechanism was deemed to be autoregulation, since it is operational even in hypophysectomized animals. Autoregulation affects both the iodide transport mechanism of the thyroid and intermediate thyroid metabolism, increasing hormone synthesis when gland stores become depleted.

High plasma levels of iodide can also alter thyroid metabolism. Large doses of iodide cause a decrease in thyroidal iodide uptake, a decrease in organic binding of iodine, and a marked decrease in thyroid hormone release (Ingbar and Woeber, 1974). In the rat, plasma iodide levels on the order of 30 mg/100 ml block thyroid iodine organification (the binding of iodine to an organic molecule), although the iodide concentrating mechanism is not immediately affected at this level. However, prolonged (16-40 hr.) plasma iodide levels of this magnitude or larger will cause the gland to lose its ability to concentrate iodide. This in turn allows organification to resume, presumably due to the lowering of the thyroid iodide concentration (Bastomsky, 1974).

b. Thyroid hormone synthesis

This subject is discussed in detail in several chapters of the review edited by Greer and Solomon (1974).

Hormone synthesis in the thyroid can be considered to begin with the trapping of plasma iodide by the gland. The mechanism of the trap is not known precisely. The most generally accepted hypothesis is that the iodide trap involves an ion pump (Wolff, 1964). The pump's effectiveness in removing iodide from the blood increases as the iodine content of the diet decreases. Studer and Greer (1968) showed that the 4-hour uptake of a tracer dose of ^{131}I rose from 4.7% in rats fed a normal diet to 64% in rats fed an iodine deficient diet for 18 days.

After iodide is trapped by the thyroid, it is oxidized to iodine, by a peroxidase enzyme. The iodine then reacts with the tyrosyl group of the amino acid tyrosine, to form mono-iodotyrosine. The tyrosine being iodinated is already a part of the storage glycoprotein thyroglobulin, which consists of 4 polypeptide chains, containing approximately 5,000 amino acids. Thyroglobulin's molecular weight is on the order of 670,000, of which approximately 8-10% is carbohydrate. About 115 of the approximately 5,000 amino acids making up the protein portion of thyroglobulin are tyrosine residues. Of these, only 10% are iodinated, and from the various forms of iodinated tyrosines, only 1-3 molecules of thyroid hormone are released when the thyroglobulin molecule is hydrolyzed (Tepperman, 1973).

The sequence of thyroglobulin synthesis is: 1) polypeptide chains and carbohydrate moieties are simultaneously produced; 2) the two components are linked to form the pre-iodinated thyroglobulin molecule; 3) some of the tyrosine residues in the thyroglobulin molecule are iodinated. This iodination may be either mono- or di-iodination; 4) in certain areas of the thyroglobulin molecule, iodinated tyrosine residues are located close enough to each other to allow condensation or coupling, forming di-tri-and tetra-iodothyronine. The post-coupling entity is the completed thyroglobulin molecule, and as such it is stored in colloid form in the central lumen of the follicle (Ui, 1974).

As surveyed by Greer and Haibach (1974), thyroid hormone secretion begins by the taking up of a drop of colloid by an epithelial cell. The thyroglobulin is acted upon by a protease, causing proteolysis of the molecule, and release of all the iodinated moieties. T_3 and T_4 are released to the blood stream, while the mono- and di-iodotyrosines are deiodinated within the cell by a deiodinase, in effect causing a recycling of a commodity scarce and important to the organism's chemical economy. Experiments indicate that the deiodinase is somewhat specific, acting to a greater extent upon iodotyrosines than iodothyronines. T_4 is secreted in greater quantities than T_3 is, although the relative amount of T_3 increases under conditions of iodine deficiency (Cacicedo, et al., 1977).

c. Thyroid hormone levels and transport in blood

Once in the blood stream, thyroid hormones are nearly totally bound to plasma proteins. In man, the important binding proteins (and the relative amount of T_4 they normally bind) are: thyroxine-binding globulin (TBG, 75%), thyroxine-binding pre albumin (TBPA, 15%) and thyroxine-binding albumin (TBA, 10%). Approximately 0.03% of the circulating T_4 is in the unbound, or free form, which is the active form of the hormone (Woeber and Ingbar, 1974). In rats, the data on T_4 binding proteins are not as clear (Nunez et al., 1965; Farer et al., 1962; Refetoff et al., 1970; Davis et al., 1970). Although different methods of chromatography give different results, the data suggest that a TBG type molecule does not exist in the rat. It is, however, generally accepted that a TBPA moiety does exist, which binds 55% of a tracer dose of T_4 , making it the most important T_4 binding plasma protein in the rat (Davis, 1970).

Thyroxine hormone levels in 2 to 4 month old female rats have been measured by Rapp and Pyun (1974), Fukuda and co-workers (1975), and Kieffer and co-workers (1975). The strains used were Sprague-Dawley rats (Fukuda), an F2 cross of Wistar and spontaneously hypertensive rats (Rapp), and Charles River (CD) rats (Kieffer). Fukuda et al. (1975) found 6.0 $\mu\text{g}/100\text{ ml}$ serum T_4 . Rapp and Pyun (1974) and Kieffer et al. (1975) reported plasma T_4 values of 6.6 $\mu\text{g}/100\text{ ml}$ and 3.9 to 5.1 $\mu\text{g}/100\text{ ml}$, respectively. Kieffer reported that female T_4 levels were slightly greater than the male levels, while Rapp reported that male T_4 levels were significantly higher than the

female levels. Riesco et al. (1977) and Lu (1976) measured values of T_4 levels over long periods of time in male rats. In both cases, a drop in serum T_4 levels from 5 $\mu\text{g}/100\text{ ml}$ at 2 months of age to $\sim 3\text{ }\mu\text{g}/100\text{ ml}$ at 1 year of age was reported.

d. Thyroid hormone action

Thyroid hormones are known to affect several processes, including growth, development, differentiation, and metabolism (Ingbar and Woeber, 1974). In addition, thyroid hormones alter the actions of other hormones, and of several organ systems, particularly the cardiovascular system (Tepperman, 1973). The importance of thyroid hormone (and other hormones which act as systemic integrators) is such that abnormal hormone levels can cause changes at every level of biological organization, from the whole organism down to the macromolecular level. Currently there is no comprehensive hypothesis which explains all the actions of thyroid hormone. Present information suggests that thyroid hormone binds to nuclear receptors in target cells. The nuclear binding modifies protein synthesis in such a way as to regulate a wide range of cell functions (Ingbar and Woeber, 1974; Edelman, 1974; Tata, 1974).

It is unclear at the present time whether both T_3 and T_4 by themselves are capable of producing thyroid hormone effects. Nonthyroidal conversion of T_4 to T_3 has been observed, as have cellular T_3 receptors. It is possible therefore that the main function of T_4 is to act as a prohormone for T_3 .

e. Goitrogens

As the term is used here, goitrogens are drugs which block thyroid hormone synthesis, and hence secretion. As reviewed by Yamada and co-workers (1974), two major classes of anti-thyroid agents exist: blockers of iodide uptake, and blockers of iodine organification. Some evidence also points to the existence of a third type of goitrogen, i.e., compounds which act by increasing excretion (fecal loss) of thyroid hormone.

Iodide uptake blockers include perchlorate, thiocyanate, and fluoborate. In addition to blocking the iodide pump, high concentrations of these compounds block organic binding of iodine by the thyroid. Additionally, it has been shown that thiocyanate discharges iodide already accumulated in a blocked thyroid gland, a property which allows clinical testing for a block of organic iodine binding in a disease state. Besides high levels of iodide which transiently block organification, certain aromatic and some thionamide compounds exhibit iodine organification blocking. The two most important thionamide compounds are 6-propyl-2-thiouracil (PTU) and 1-methyl-2 mercapto-imidazole (MMI, or methimazole).

The mechanism of goitrogen action is not well understood. According to Yamada et al. (1974): "Most experimental evidence supports the theory that anti-thyroid drugs act through their reducing action ... However this reducing theory is not satisfactory, since many reducing substances such as cysteine, glutathione, and thioglycollic acid do not interfere with thyroid hormone synthesis,

although their ability to reduce iodine is similar to that of thiourea". A second possible mechanism of goitrogen action is inhibition of a specific enzyme. The peroxidase involved in the iodide to iodine conversion has been suggested as one possible candidate (Yamada et al., 1974).

Methimazole has been shown to cause significant effects in rats when given in high doses. Owens and co-workers (1973) placed male and female weanling Harlan rats on rations of 0, 5, 30, or 180 parts per million (ppm) MMI mixed into the diet. After two years, the survivors were sacrificed. The 180 ppm animals exhibited reduced longitudinal growth, but became obese at maturity. They also exhibited a higher incidence of fatty degeneration in the kidneys and liver. Mortality was increased at the 180 ppm level, being 50% at 1 year and 94% at 2 years, vs. 10% and 80-84% at 1 and 2 years, respectively, for the other groups. Thyroid enlargement, including hypertrophy and hyperplasia, was evident in the 30 and 180 ppm rats, but not in the 5 ppm rats. An increased number of follicular adenomas and adenocarcinomas was seen in the 30 and 180 ppm animals. The incidence data, as expressed by Owen et al. in terms of number of rats with tumor/number of rats surviving when the first tumor was detected in a group, are given below.

<u>Tumor Type</u>	<u>Methimazole level in diet - ppm</u>			
	0	5	30	180
Follicular Adenoma	1/55(2%)	1/8(12%)	31/55 (56%)	17/32 (53%)
Follicular Adenocarcinoma	1/17(6%)	0 (0%)	5/42 (12%)	5/24 (21%)

There was no indication of any sex difference in the incidence of either type of tumor. The incidence and time of onset of light cell tumors (the cellular origin of which are presumed to be thyroidal C-cells) were reported to be unchanged by treatment with MMI.

Metabolism of MMI was investigated by Marchant and Alexander (1972), who reported that the majority of radioactivity excreted by male Sprague-Dawley rats within four days after an intraperitoneal injection of 134 mg of ^{35}S -methimazole ($\sim 40 \text{ m Ci/m mole}$) was found in the urine (64% of administered dose), while 7.8% of the dose appeared in the feces. Approximately 1% of the administered dose was found in body tissues. The lack of accountability for the remainder of the radioactivity ($\sim 27\%$) was ascribed to the method of urine and feces collection. On the basis of tissue distribution and chromatographic studies these authors postulated that the methimazole was primarily oxidized in the thyroid and excreted by the kidney.

The results of Marchant and Alexander (1972) agree with those of Pittman et al. (1971), who studied the metabolism of 3 mg of ^{14}C -MMI given intravenously to male Sprague-Dawley rats. After four days 76% of the radioactivity had appeared in the urine and 6% in feces. Approximately 8% was found in body organs. An interesting finding by Pittman et al. was that on a per-gram basis, at 4 days after intravenous administration, the adrenals had nearly as much radioactivity as the thyroid did, and much more activity than any other organ (thyroid 2.2, adrenal 1.7, other organs 0.45 $\mu \text{ moles/gm}$).

It has also been reported (Hashizume and Yamaki, 1977) that MMI can reduce nor-adrenaline content in the rat brain at 1 to 6 hours post-administration when given in high doses (25 or 50 mg/kg intra-peritoneally). Whether this has any effect on hypothalamic TRH function is unknown.

3. Thyroid tumor classification

One of the most detailed descriptions of rat thyroid tumors is found in the work of Axelrad and Leblond (1955). Although the tumors they characterize arose as a consequence of a low iodine diet, the descriptions are applicable to radiation-induced tumors as well (Lindsay, 1969; Doniach, 1970). However, since Axelrad and Leblond used a tumor classification system of α , β , γ , with sub-categories in each category, rather than conventional adenoma/carcinoma terminology, their classification scheme is infrequently used. Papers using the conventional adenoma-carcinoma characterization scheme include those of Lindsay et al. (1966, 1968a), Lindsay (1969), and Doniach (1970, 1974).

Certain types of spontaneously occurring rat thyroid tumors, called "natural" tumors (also called microadenomas; light cell adenomas; nodules; alveolar, lobular, solid, or medullary carcinomas) were not identified as being spontaneous in origin until 1957 (Lindsay et al., 1957), although their morphology is distinct from TSH related tumors. As a result of their separate (and unknown) etiology, these tumors have usually been excluded from analysis of data on radiation-induced tumors. The incidence of "natural" tumors increases markedly

with age (Lindsay et al., 1968b). According to Doniach (1970), the incidence of this particular type of tumor is reduced in goitrogen-treated rats, as well as in heavily irradiated rats. Lindsay and co-workers (1968b) reported that low doses of ^{131}I (1, 10, 25, or 40 μCi) do not significantly alter the incidence of "natural" carcinomas. However, in another paper in the same year (Lindsay et al., 1968b), they report that 5 μCi of ^{131}I increased the incidence of natural tumors as compared to unirradiated controls (7/52 vs. 0/40, $\chi^2: P < 0.25$).

In reporting tumor incidence in tabular form, Doniach (1950, 1953, 1957, 1974) generally referred only to adenomas and carcinomas, without further classification. However, in the body of these reports, Doniach described morphologically different adenomas and carcinomas. Lindsay et al. (1966, 1968a) also did not make a distinction among types of adenomas, although they differentiated between follicular and papillary carcinomas.

The distinction between adenoma and carcinoma is not always an easy one to make (Lindsay, 1969). Doniach (1974) indicated the following criteria for carcinoma identification: "Thyroid carcinomata were more readily identified by their invasive properties than by any specific cellular morphology or arrangement. The diagnosis of malignancy was restricted to neoplasms showing clearcut transgression of capsule and/or permeation of the lumen of capsular venous sinusoids or of extra-thyroidal veins."

Lindsay and co-workers (1966) used a less stringent definition of carcinoma, accepting as carcinomas tumors which "were circumscribed

but unencapsulated nodules ... Varying degrees of invasion of the adjacent thyroid parenchyma or the presence of small satellite nodules represented the only aggressive features ... Neither vascular nor lymphatic invasion was observed." Lindsay et al. (1966) also did not make a distinction among types of adenomas, although they did differentiate between follicular and papillary carcinomas.

Thyroid adenomas have been described as circumscribed, encapsulated, and sources of compression and atrophy for the surrounding parenchyma. According to Lindsay (1969), the tumors of animals on goitrogen treatment often evidenced "extreme prominence of dilated thin-walled venous sinuses between the neoplastic cells. The sinuses frequently enlarged to form venous lakes which became the site of thrombosis, organization, and in some instances, calcification. ... The adenomatous cells were uniform, usually columnar, with oval or round compact nuclei lying near the basement membranes. There were no mitotic figures." Carcinomas, according to Lindsay (1969), ranged from well differentiated forms to tumors with pleomorphic hyperchromatic nuclei. Mitotic activity ranged from insignificant to high. Lindsay also reported that most papillary carcinomas developed as malignant neoplasms from their inception, but a few originated in follicular adenomas (Lindsay, 1966), while the majority of follicular carcinomas originated in pre-existing benign adenomas (Lindsay, 1969).

B. Radiation-Induced Thyroid Tumorigenesis In Man

Data exist which show that irradiation of the thyroid in man increases the risk of developing thyroid tumors. The data have been collected from three distinct populations: 1) the survivors of the atomic bomb explosions at Hiroshima and Nagasaki; 2) inhabitants of the Marshall Islands who were accidentally exposed to fall-out from a nuclear weapon test in 1954; and 3) medical patients whose thyroids were exposed to ionizing radiation as a consequence of treatment for various pathologic conditions.

The 1972 Report of the BEIR Committee of the National Academy of Sciences (NAS-1972) reviewed the thyroid tumor incidence of these three populations and estimated an upper limit of risk of thyroid tumor development of the order of 1 to 10 cases per year per 10^6 children exposed per rem. The BEIR report pointed out that thyroid tumor incidence in adults, as found in two different studies, is not significantly elevated, even at high dose levels of radiation.

According to this report: "These studies confirm the lesser susceptibility of the adult thyroid to radiation-induced carcinogenesis, as compared with the thyroid of the infant or child." More recent publications dealing with the same exposed populations include the annual reports of the Radiation Effects Research Foundation (RERF), the report edited by Conard (1975) on the Marshall Island population, and the reports on the medically exposed people by Hempelmann et al. (1975), Greenspan (1977) and Refetoff et al. (1975).

Malone (1975) briefly surveyed the available data on the carcinogenic effects of radiation in the human thyroid in his review of the radiation biology of the thyroid. In considering possible mechanisms of thyroid tumor formation following irradiation Malone stated: "Despite a definite association between irradiation and benign and malignant thyroid tumors in persons whose gland was irradiated during childhood, there are reasons for believing that irradiation is not the only factor involved (Hemplemann, 1969). In particular there is a lack of correlation between thyroid dose and the latent period between exposure and appearance of the tumor. Furthermore there is evidence to suggest that tumours tend to occur at the same age, regardless of whether irradiation took place in early or late childhood. There is also a lack of correlation between dose and the type of tumour which develops (Hemplemann et al., 1967; Wood et al., 1968)". Malone then considered an additional factor by referring to animal experimentation and theories that have been derived from animal work. "These theories postulate that lasting primary radiation injury to thyroid cells may lead to tumor development, as a result of growth of the gland and TSH stimulation. ... The increased TSH output during puberty and normal growth and maturation of the gland, contribute the other factors necessary to explain the development of tumors as a result of thyroid irradiation during childhood (Doniach, 1971; Walinder, 1973; Hemplemann, 1969)".

C. Radiation-Induced Thyroid Tumorigenesis In Experimental Animals

1. Introduction

Animal experiments designed to investigate the radiation induction of thyroid tumors have utilized rats, mice, dogs and sheep. The animal experiments have involved the use of internal radioactive isotopes (^{125}I , ^{131}I , ^{211}At) or X-ray exposure. At the time of exposure, the animals have been mature, adolescent, neonatal, or fetal. Both male and female animals have been used. Radiation has been administered alone or in combination with other agents, mainly goitrogens (Methylthiouracil, propylthiouracil or aminotriazole), or an iodine deficient diet. Reviews of such investigations include those of Lindsay (1969), Doniach (1970), and Malone (1975).

2. Rat studies

One of the first reports concerning radiation tumorigenesis in rat thyroid was that of Doniach (1950). In this study, 32 μCi of ^{131}I given intraperitoneally (i.p.) was administered alone or in combination with: 1) methylthiouracil (MTU); 2) acetylaminofluorene (AAF), a known carcinogen; or 3) AAF plus MTU. The rats were examined for thyroid tumors 9 to 17 months post-exposure.

The tumor incidences were as follows: Control, 4/15; ^{131}I only, 10/16; MTU only, 10/16; MTU+ ^{131}I , 5/5; AAF alone, 8/12; AAF+ ^{131}I , 3/5; AAF+ ^{131}I +MTU, 6/6. All the treatments increased the fractional tumor incidence over that of the control rats (χ^2 , $p < .05$). Combining MTU and ^{131}I increased the fractional tumor incidence, as compared to the single treatment incidences although the increase is not statistically

significant (χ^2 , $p > .05$). Combining AAF and ^{131}I did not cause an increase in the fractional tumor incidence as compared to the incidence of tumors from either treatment given singly. The only thyroid carcinoma found in the 120 animals examined was in an animal that received AAF+MTU+ ^{131}I .

Doniach (1953) then injected rats with 5, 30, or 100 μCi of ^{131}I alone or in combination with MTU feeding for 15 months. The 5 and 30 μCi ^{131}I treatments alone caused increases in thyroid tumor incidence above that in the nonirradiated control group, but the thyroid tumor incidence in the 100 μCi ^{131}I groups was the same as that in the non-irradiated control group. This may have been due to massive reproductive sterilization and killing of thyroid epithelial cells at this high radiation level. All of the ^{131}I +MTU groups had tumor incidences greater than their respective irradiated (non-MTU) control groups. The only malignancies found were in the 30 μCi ^{131}I +MTU group, where 5 of 20 animals had carcinomas.

A comparison of the thyroid tumorigenic effects of 1100 rads of 200 kVp X-rays and 30 μCi of ^{131}I was made by Doniach in 1957 (Doniach, 1957), again using treatments of radiation alone and radiation+MTU. Of the rats receiving X-irradiation alone, 4 of 13 developed adenomas. One X-ray-only rat developed a carcinoma. In the X-ray+MTU group 21 of 22 rats developed adenomas, and 7 of 22 rats had carcinomas. In the ^{131}I -only group, 4 of 22 rats had adenomas, and in the ^{131}I +MTU group 23 of 24 rats had adenomas and 5 of the 24 rats had carcinomas. Doniach estimated that the radiation dose to the rat thyroid from 30 μCi of ^{131}I

was of the order of 10,000 to 15,000 rads. On the basis of tumor producing potential in the rat thyroid, he estimated that 30 μ Ci of ^{131}I was roughly equivalent to 1000 rads of X-rays.

In 1974, Doniach reported a study of the thyroid tumorigenic effects of thyroid X-irradiation (100; 250; 500 rads) alone or in conjunction with administration of either thyroxine (5 mg/100 gm) or aminotriazole (ATA). The rats were sacrificed 18 to 20 months post-exposure. All of the ATA-treated rats developed thyroid adenomas. The thyroxine+radiation groups had only 1 adenoma in 71 rats, while the radiation-only groups had 5 adenomas in 86 rats, suggesting that thyroxine administration reduced the number of tumors produced after irradiation. However, this reduction is not statistically significant (χ^2 , $p > .10$).

The results of Doniach's experiments, as interpreted by Lindsay (1969), show that: "...either ^{131}I or X-rays caused irreversible changes in thyroid epithelial cells, rendering them susceptible to neoplastic development when stimulated to become hyperplastic. Thyrotropic stimulation, either goitrogen-induced or resulting from interference with thyroxine formation caused by radiation damage, or both, (may be) regarded as a factor promoting thyroid neoplasia."

Goldberg and Chaikoff (1952) reported the development of benign and malignant thyroid tumors in rats 1-1/2 to 2 years after administration of 400 μ Ci ^{131}I . In approximately 1/3 of the rats injected with 400 μ Ci of ^{131}I , the thyroid was ablated as a result of the radiation received. It was later reported that many of the tumors found in this experiment were of the type which occurs naturally (Lindsay et al., 1968b).

These investigators (Lindsay et al., 1957) did further studies using smaller doses of ^{131}I (10-400 μCi), and determined that thyroid adenomas were found most frequently in the rats given the lower (10 or 25 μCi) doses of ^{131}I . These experiments also suggested that in rats given low doses of ^{131}I , females have a lower incidence of thyroid carcinomas than males, the reverse of the situation for naturally occurring human carcinomas (Lindsay et al., 1963).

Lindsay and co-workers (1966) then gave 25 μCi of ^{131}I to rats, with or without adjunctive treatment with dessicated thryoid powder (DTP) and/or PTU. At 12 months after ^{131}I injection, the incidence of thyroid tumors in the ^{131}I -only and the ^{131}I +DTP groups was the same (10%). The PTU-only group had a tumor incidence of 48%. The ^{131}I +PTU and PTU+DTP groups both had 65% incidence of thyroid tumors, and the ^{131}I +PTU+DTP group had a 78% thyroid tumor incidence.

This same group of investigators also investigated the thyroid tumorigenic effect of the combination of ^{131}I irradiation and subtotal thyroidectomy. The incidence of thyroid adenomas was greater in the group receiving 1 μCi ^{131}I in conjunction with subtotal thyroidectomy than in the group which was subjected only to bilateral lobectomy. The addition of DTP feeding to the two types of treatments (^{131}I +subtotal thyroidectomy or thyroidectomy), reduced the incidence of thyroid tumors (Goldberg et al., 1964). This again fits the hypothesis that TSH stimulation of the thyroid (increased by the thyroidectomy and decreased by the DTP feeding) is important for thyroid tumor formation.

In 1968, Lindsay reported on a further study in which rats were injected with 1 or 5 μCi of ^{131}I and examined at 0.5, 1 and 2 years post injection. At two years post-injection, the tumor incidence for the 1 μCi group was not significantly different from that of the nonirradiated control group, (2% vs. 0%), while the incidence in the 5 μCi group was significantly greater (12%, $p < .025$) than that in the control group. The thyroids in the 5 μCi group displayed no histologic evidence of either radiation injury or excessive TSH stimulation, indicating that changes more subtle than those which can be detected histologically are capable of being tumorigenic (Lindsay et al., 1968a).

Earlier, Lindsay and co-workers (1961) used X-rays to induce thyroid tumors. In an interesting variation on what had previously been done, they attempted to irradiate only one lobe of the rat thyroid (1000R) by shielding the other lobe with lead foil. Hyperplasia occurred in the shielded lobe in most of the rats, presumably from TSH stimulation caused by the radiation effect on the irradiated lobe. Since much less radiation damage existed in the shielded lobe, it was capable of a greater hyperplastic response than was the irradiated lobe. Fewer adenomas occurred in the shielded lobes than in the unshielded lobes, showing that thyroid tumors post-irradiation do involve a direct effect of radiation and do not arise solely from an indirect (abscopal) radiation effect. The degenerative and Askanazy changes, defined by Lindsay (1969) to be hyperchromatism and pleomorphism of cell nuclei together with abundant vacuolated cytoplasm, seen in other rats in this experiment, which received 500,

1000, or 2000R to the entire thyroid, were reported as being roughly equivalent to those changes seen in rats receiving 10 to 40 μCi of ^{131}I . This agrees well with Doniach's (1957) estimate that 30 μCi ^{131}I had the same effect on thyroid tumor formation as did 1000R of X-rays.

Carrying the single thyroid lobe irradiation work one step further, the Nichols et al. (1965) reported on the action of DTP in conjunction with hemi-thyroid X-irradiation (1000R). Two years post-irradiation 15% of the radiation-only animals had thyroid tumors, while no animals of the 1000R+DTP group had thyroid tumors. This added support to the argument that TSH stimulation plays a part in thyroid tumor formation.

Other researchers have also investigated the thyroid tumorigenic response to radiation in rats. Frantz and co-workers (1957) used 400 μCi of ^{131}I or 900 rads of X-rays. As happened to Goldberg and Chaikoff (1952), the level of radiation from 400 μCi ^{131}I was so great as to almost totally destroy the thyroid gland. Only 2% of these rats developed adenomas and none developed carcinomas. Only a small fraction of the X-irradiated rats developed thyroid tumors (2% with adenoma and 9% with carcinoma, as compared to 30% and 7%, respectively, reported by Doniach (1957) for 1100R of X-rays.) Field and co-workers (1959) also reported the failure of a large dose of radioiodine to produce rat thyroid tumors.

Nadler (1969) reported that 300 rads of X-rays, when followed by an iodine deficient diet, was able to produce significant numbers of

thyroid tumors in only 9 months. This indicates that promotion of radiation-related thyroid tumors can arise not only as the result of goitrogen administration, but also as a consequence of iodine deficiency. Nadler and co-workers (1970) carried their work further by comparing thyroid tumor incidence among groups of rats which received some combination of 3 μ Ci of ^{131}I , a low iodine diet, (I^-), hypophysectomy, or sham-hypophysectomy. As opposed to the majority of the previous studies, these rats were female. In addition, they were younger than rats used in previous experiments, being only 10 weeks old at the time of ^{131}I injection. The survivors were sacrificed at 6 months post injection. The rats which were hypophysectomized had no thyroid tumors, no matter what other treatments (I^- , ^{131}I) they received. Iodine deficient rats that were not hypophysectomized had a thyroid tumor incidence of 54%. Rats that were not hypophysectomized but which received both a I^- diet and ^{131}I had a tumor incidence of 98%. The rats receiving ^{131}I only developed no tumors. These investigators concluded that "the anterior pituitary gland; presumably through the secretion of TSH, is prerequisite for the development of neoplasm of the thyroid follicular cells...".

Christov (1975) reported on work in which 3-month old male Wistar rats were given 300 rads of X-rays to the thyroid 1 day before, or 1, 8 or 20 days after, the onset of MTU administration. In the three cases where irradiation was performed after MTU treatment was begun, the groups were divided into two subgroups. One subgroup was taken off MTU treatment at the time of irradiation, while the other

subgroup was kept on MTU treatment for the remainder of the experiment (15 to 18 months).

Christov had previously determined that in Wistar rats, continuous MTU treatment (0.1% in drinking water) causes the thyroid to grow "exponentially". The "exponential" growth starts two days after the beginning of the MTU treatment, and lasts until nine days after the beginning of the MTU treatment. Slower growth continues from nine days after treatment out to at least 24 days after treatment. By irradiating at days +1, +8, and +20 with respect to the onset of MTU treatment, the thyroid was irradiated just before the growth spurt, during the growth spurt, or during the stage of slower growth. The tumor incidences in animals maintained 18 months on MTU and irradiated on day -1, +1, +8 and +20 were 69, 83, 100, and 90% respectively. The tumor incidences for animals taken off MTU after irradiation on day +1, +8, and +20 were 30, 75, and 62% respectively.

Lu (1976) reported that 9 of 10 rats sacrificed at 21 months after an X-ray exposure of 1300R to the head and neck region showed thyroid tumors. Three of the animals had only medullary type tumors, leading Lu to state that, "The prevalence (of thyroid tumors related to X-irradiation of the thyroid gland) at this age period was thus 6 out of 10 rats sacrificed."

At 24 months post-exposure, he found only one thyroid tumor (medullary tumor) in the six rats sacrificed. At 27 months post exposure, one of the three rats sacrificed had a thyroid medullary tumor, one had a thyroid adenocarcinoma, and one had both a medullary

tumor and an adenocarcinoma. When comparing the tumor incidences in Lu's experiments with tumor incidences from other experiments it should be kept in mind that Lu irradiated the head and neck of his rats, so that the pituitary was directly irradiated, while the other experiments discussed used neck only irradiation. While neck only irradiation does not totally protect the pituitary from radiation, it does lower the dose to the pituitary to approximately 20% of the thyroid dose (see Methods and Materials).

Radiation tumorigenesis in thyroid of neonatal rats was investigated by Doniach (1969). Male and female rats less than 5 days old were given 1.1, 2.9, or 5.5 μCi of ^{131}I subcutaneously (S.C.). One year later, the 115 experimental animals and 20 untreated controls were examined for thyroid tumors. The tumor incidences found were 0/20, 9/35, 13/33, and 13/27 for the 0.0, 1.1, 2.9, and 5.5 μCi groups, respectively. At the 5.5 μCi level, males had a significantly higher tumor incidence than did females (11/14 vs. 2/13). At the other two dose levels, tumor incidence in males was slightly higher than in females, but not significantly so (43 vs. 37%, and 29 vs. 21%). Doniach suggested that the greater male tumor incidence could be related to the fact that when compared to female follicular cells, male follicular cells at each dose level had a greater mean cell height, suggesting greater TSH stimulation. The tumor incidence among the neonates given 2.9 μCi ^{131}I was approximately equal to that seen in adult rats given 30 μCi (Doniach 1953, 1957). By taking into account variation between adult and neonatal rats in

iodine uptake, iodine retention, thyroid gland mass, and thyroid gland size as related to ^{131}I β radiation range, Doniach arrived at the conclusion that the radiation doses in the adult 30 μCi and neonate 2.9 μCi groups were approximately the same, implying that no difference exists in thyroid tumor susceptibility between the two groups.

3. Studies in Other Experimental Animal Species

Animals other than rats have been used to study radiation related tumors.

Walinder and Sjoden (1972a, 1972b, 1973) have reported on thyroid irradiation experiments using mice. In an attempt to compare both age differences and radiation differences, they used fetal and adult CBA mice, with injected ^{131}I and X-rays from an external source as the sources of radiation. Adult mice (96 days old) were injected with ^{131}I and sacrificed at 374-376 days of age. The estimated thyroid radiation dose was 8500 to 9500 rads. No thyroid tumors were observed in 91 animals. Fetuses were exposed at the 18th day of gestation to an estimated thyroid dose of 2400, 4700 or 7800 rads of ^{131}I radiation. At 323 to 348 days of age the fetal ^{131}I exposed rats were sacrificed. No tumors were seen in the lower two dose groups, while the 7800 rad groups had a tumor incidence of 4% for females and 10% for males. Fetal exposure to 180 rads whole body X-irradiation alone or in combination with 2400 rads (thyroid) of ^{131}I produced no tumors at 323 to 348 days of age. However, 180 rads whole body X-irradiation at the 18th day of gestation in

combination with 4700 rads (thyroid) of ^{131}I produced a 7% thyroid tumor incidence in females and a 0% thyroid tumor incidence in males at 323 to 348 days of age.

Reports of radiation-induced dog thyroid tumors have been published by Andersen (1964) and by Lu and co-workers (Lu et al., 1973).

Marks and Bustad (1963) reported thyroid neoplasms in sheep fed 1.5 to 135 μCi ^{131}I daily. Animals fed 1.5 or 5.0 μCi per day received the dose from fetal age throughout life, while the animals receiving 45 or 135 μCi daily were exposed only during adult life (15 months of age and beyond).

II. PURPOSE AND SPECIFIC OBJECTIVES

The overall purpose or aim of this study was to investigate the relationship between the variation in thyroid radiation (X-ray) dose, over a range of moderate doses, and the variation in dosage level of chronic goitrogen treatment, in the induction of thyroid tumors.

The specific objectives of the study were to determine:

1. The thyroid tumor incidence in rats receiving various doses of a goitrogen chronically after localized thyroid X-irradiation at various dose levels;
2. whether at the various levels the two treatments (localized X-irradiation and goitrogen administration) interact in any way to affect thyroid tumor incidence, and if so, whether the interaction is less than additive, additive, or synergistic (greater than additive);
3. both short-term and long-term effects of the treatments on the serum thyroxine response;
4. semi-quantitative and quantitative histological changes in the thyroid following the treatments.

III. METHODS AND MATERIALS

A. Experimental Design

Three distinct experiments were performed.

1. Experiment I

Experiment I ran for 13 months post-irradiation, and consisted of 15 experimental groups formed by combining 5 thyroid radiation dose levels (0, 80, 160, 320, 640 rads) with three levels of methimazole (MMI) in the rats' drinking water: 0 parts/million (ppm); 4 ppm (low); and 40 ppm (medium). Due to logistical constraints, primarily in animal breeding and maintenance facilities, the more than 700 animals used for this experiment were entered into the experiment in 4 batches (A, B, C, D) at 4 separate times. The numbers of rats per experimental group for each batch are shown in Table 1.

2. Experiment II

Experiment II ran for 7-1/2 months post-irradiation and consisted of five experimental groups: 0, 80, 160, 320 or 640 rads thyroid radiation dose combined with a high MMI level (200 ppm) in the drinking water (Table 1).

3. Experiment III

Experiment III was designed to measure serum thyroxine (T_4) levels within the first three months post-irradiation. This experiment consisted of nine experimental groups (4 rats in each group), formed by combining two thyroid radiation dose levels (0 rads and 640 rads) with the three levels of methimazole (0, 4 or

TABLE 1
NUMBERS OF RATS IN EXPERIMENTS I, II, AND III

		Thyroid Radiation Dose (rads)					
Batch	MMI (ppm)	0	80	160	320	640	Totals
Experiment I							
A	0	25	28	22	26	25	
A	4	21	20	21	21	20	
A	40	19	20	21	20	20	
B	0	3	3	3	3	0	
B	4	5	9	10	8	0	
B	40	6	6	11	6	0	
C	0	15	17	17	11	17	
C	4	3	9	9	3	12	
C	40	9	6	9	5	6	
D	0	5	5	6	10	10	
D	4	21	9	6	12	20	
D	40	15	11	10	15	8	
A+B+C+D	0	48	53	48	50	52	251
A+B+C+D	4	50	47	46	44	52	239
A+B+C+D	40	49	43	51	46	34	223
(Totals)	-	147	143	145	140	138	713

Experiment II

-	200	34	34	32	35	35
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Experiment III

-	0	4	0	0	0	4
-	4	4	0	0	0	4
-	40	4	4	4	4	4

40 ppm MMI) in drinking water, and three thyroid radiation dose levels (80, 160, 320 rads) with 40 ppm MMI in drinking water (Table 1).

B. Animals and Their Maintenance

All experiments were done using Rochester ex-Wistar female rats, three to five months old in Experiments I and III, and three to six months old in Experiment II. The animals were marked by ear punching, weighed, and divided randomly into experimental groups. Animal weights ranged from 190 to 250 grams at the start of Experiments I and III, and from 190 to 280 grams in Experiment II. The rats were housed two or three to a cage at $72^{\circ}\text{F} \pm 2^{\circ}\text{F}$ in rooms with a 12 hours on (0600 - 1800 EST), 12 hours off, light cycle. Due to spontaneous deaths, periodic consolidation of rats was required to maintain the two or three to the cage environment. Pelleted Agway rat chow was provided ad libitum to all animals. Tap water, or tap water containing dissolved Methimazole, depending on the experimental group, was also provided ad libitum.

C. Radiation Factors and Procedures

X-irradiation was performed using a General Electric Maxitron II X-ray unit. The exposure parameters were: 300 kVp, 20 mA, TSD 35 cm, 2 mm Cu added filtration.¹ These values produced an exposure rate of 62R/15 sec $\pm$ 7R in air as measured by a Victoreen 100R chamber when placed in the part of the exposure field to be occupied by the rat thyroid.

Thermoluminescent dosimeter cylinders (TLDs) (1.5 mm i.d.) containing 30 mg LiF were used to determine absorbed doses at various

¹HVL=1.6 cm Cu

points in and about the body of a sacrificed rat. The light output of the TLD's was measured using a Harshaw 2000A reader and 2000B oven operating in the standard heating mode.

A curve relating exposure and absorbed dose in air was created by placing TLD's in air and irradiating them for varying periods of time. The amount of light emitted by an exposed TLD when heated is proportional to the amount of energy the TLD has acquired as a result of exposure to ionizing radiation. Plotting, for various exposures, the exposure dose in Roentgens, as measured by a Victoreen R chamber, against the light emitted by a TLD from the same exposure generates the calibration curve. Multiplying the air exposure dose by a conversion factor of .95 gives the absorbed dose (rads) in tissue. Using TLDs, it was determined that the thyroid, pituitary and upper breast received 80, 17, and 3% of the neck surface exposure. Thus, for every 100 Roentgens exposure to the neck, the thyroid and pituitary received 80 and 16 rads of absorbed dose, respectively, indicating that the pituitary's absorbed dose was 20% that of the thyroid.

Irradiation was performed on five animals at a time. The animals were placed ventral surface up on a turntable which was rotated (.9 rpm) during irradiation in order to even out inhomogeneties in the radiation field. Each animal was placed in a 1" thick lead cylinder, which shielded the body from the calvicle to a point approximately near the hind leg - pelvic girdle joints, the exact location depending on the size of the rat. The animal's head was

shielded by a 1/4" thick lead shield which covered the head from the nose down to the symphysis of the mandible.

D. Anesthesia

1. For Irradiation

For irradiation, the rats were anesthetized by injecting sodium pentobarbital (Nembutal 65 mg/ml) intraperitoneally (i.p.) at a dosage of approximately 40 mg/kg body weight. All animals were anesthetized, including the sham-irradiated members of the zero radiation groups.

2. For Blood Sampling

The volatile anesthetic halothane (Fluothane, Ayerst) was used during blood sampling. Halothane was chosen as the anesthetic agent for three reasons: 1) animals become rapidly anesthetized and rapidly recover from halothane exposure (~30 sec from time of exposure to loss of blink reflex, or from end of exposure to first movements); 2) animals tolerate halothane well, showing no long term effects even after repeated exposure, in contrast with ether, which seems to cause respiratory problems or exacerbate already existing ones; and 3) animal death due to halothane exposure is very rare in the experience of this laboratory. There have been reports in the literature of focal to massive liver necrosis in human beings repeatedly exposed to halothane. The etiology of this reaction is unknown, although an immune reaction has been postulated (Robbins, 1974). No evidence of such a reaction was seen in any of the rats in this study which were histologically examined.

E. Goitrogen And Its Administration

Methimazole (MMI) (2-Mercapto-1-methylimidazole, 98%), obtained from the Aldrich Chemical Co., was used as the goitrogen because of its ease of applicability due to high water solubility and stability, and because of a lack of noticeable side effects at the dosage levels used. Methimazole water was made up in 20 liter carboys as needed, but no less frequently than once a week. Thin layer chromatography tests run before the experiments began indicated that methimazole was stable in water for at least 1 month, with no detectable breakdown products being formed.

A pilot study was run to determine the toxicity of MMI. Three groups of 20 Rochester ex-Wistar female rats 3 to 5 months old were given MMI drinking water. The levels of MMI in the water were 1,000 ppm, 200 ppm, and 40 ppm. After one month, the 1000 ppm group showed 25% mortality and an average weight loss of 15%. This group was discontinued at this point, and three animals were sacrificed, two of which showed signs of fatty liver degeneration. The 200 ppm and 40 ppm groups were continued on MMI for eight additional months, at which point the survivors were sacrificed. The thyroids of the 200 ppm rats showed tumor formation, while those of the rats in the 40 ppm group did not. Water consumption was measured in nine of the rats from each of these two groups over a period of four days. Average water consumption was determined to be 22.3 and 25.2 ml/day for the 200 ppm and 40 ppm groups, respectively. This difference in water consumption was not statistically significant as measured by

Student's 't' test ($p > .05$). Consumption of tap water by Rochester ex-Wistar female rats in the laboratory had previously been measured and found to be approximately 25 ml/day.

F. Experimental Techniques

1. Blood Sampling

After an animal was anesthetized, it was placed ventral surface up on a surgical board, with spring action clothes pins holding down the two forepaws. The cervical area to be used for bleeding was wiped with 70% alcohol. For the first seven bleeding periods of Experiment I, a skin incision ~1 cm long was used to expose the jugular vein. After the first seven bleeding periods, enough skill had been developed to allow bleeding from the jugular without skin incision, so that the remainder of the bleeding in this experiment and all of the bleeding in Experiment III was done without skin incision. Palpation allowed the location of the external jugular as it passed over the clavicle and under the pectoralis major, permitting withdrawal of blood without incision.

The right jugular vein was used for bleeding unless difficulties prevented collection (approximately 5% of the time) in which cases the left jugular vein was used. The time elapsed between removing the animal from her cage and collecting the blood varied from less than 1 to approximately 7 minutes, with the large majority of collections requiring 2 to 4 minutes. Ten to fifteen samples per hour was a typical collection rate.

Blood sample taking was restricted to the hours 10:00 A.M. to 12:30 P.M. EST to reduce possible effects of a circadian rhythm in hormone levels. For Experiment III (short-term T_4 study), blood sampling was done in one or sometimes two days for a time point. For Experiment I (the long-term study), sampling for a time point required two or three days. A 3 ml syringe with a 22 gauge needle was used to remove 1.1 to 1.5 ml of blood. After bleeding, the incision, if one was made, was sprayed with an antibacterial formulation (Neosporin) and closed with wound clips, after which the animal was returned to its cage. The blood was expressed into a 12 X 75 mm test tube and stored in a refrigerator. One hour after the collection of the last sample, all samples were centrifuged at approximately 700 g for 10 minutes. The resulting supernate (serum) was pipetted out and stored frozen at -20°C until used for assay.

2. T_4 Assay

T_4 was assayed using a commercial RIA kit (Pentax Corporation). Samples and standards were run in duplicate. For Experiment I (the long-term experiment), each month's samples were run together within a week of collection. For Experiment III (the short-term study), samples were assayed after the last collection, with all samples in a particular assay coming from the same treatment group. A standard provided by Pentax was run with each determination to provide an inter-assay calibration.

3. Sacrifice Procedures And Tissue Handling

At the end of 13 months in Experiment I, and 7-1/2 months in Experiment II, the surviving animals were sacrificed by Nembutal overdose (.5 to 1 ml/animal i.p. injection). All tissue samples taken were fixed in 10% neutral buffered isotonic formalin (NBIF) unless otherwise noted. The thyroid was removed by clipping the trachea and esophagus as close as possible to the top and bottom of the thyroid. The assembly was weighed and placed in NBIF. Cervical lymph nodes were also taken, to be examined for possible metastases. The abdominal cavity was then opened and examined for abnormalities. Samples were routinely taken from the liver, and if they appeared abnormal, of the lung, spleen, breast, adrenals, kidneys, and ovaries. The animal was then turned over and the pituitary was removed. In the latter experimental batches (C and D), the pituitary was fixed in Bouins fixative rather than formalin.

Essentially the same procedures were performed with animals which died spontaneously, unless autolysis was so severe as to preclude the usefulness of the tissues for histologic examination. All tissues were stained with hematoxylin and eosin, except the Bouin fixed pituitaries, which were stained with a combination of aldehyde-fuchsin, light green yellowish, and Orange G in order to differentiate thyrotroph cells.

Thyroids from sacrificed animals were serially sectioned at 6 μ thickness, with every 10th section being taken for examination.

4. Histology Procedures

The thyroid sections were first examined to determine tumor types and extent of tumor involvement for each sacrificed animal in Experiment I and Experiment II. The extent of thyroid tumor involvement was graded on a 0 - 4 scale as follows:

- 0 = No tumor present in the gland.
- 1 = Tumor(s) present and estimated to constitute less than 5% of the gland volume.
- 2 = Tumor(s) estimated to constitute 5 to 20% of the gland volume.
- 3 = Tumor(s) estimated to constitute 20-50% of the gland volume.
- 4 = Tumor(s) estimated to constitute more than 50% of the gland volume.

The rats were divided into six categories, depending upon the types of tumors observed in their thyroids: 1) follicular adenoma; 2) papillary adenoma; 3) mixed papillary-follicular adenoma; 4) medullary tumor; 5) medullary tumor and follicular adenoma; and 6) carcinoma.

One section from each gland was then used to quantify the relative areas of the non-tumorous thyroid gland tissue occupied by the tissue components. The section used was generally from the second fifth of the gland, as determined by counting the number of sections taken from the gland and labeled for order. A net micrometer grid was placed in one eyepiece and, using a high dry objective (total

magnification of 500 diameters), the types of tissue components present at the intersections of the crosslines were recorded.

This was done for all 49 intersections in each field quantified. A total of 10 to 13 fields per thyroid section were systematically quantified in this manner. Succeeding fields were contiguous, but not overlapping. The tissue components at the intersections of the hair lines were recorded according to the following five categories: connective tissue cells and matrix; epithelial cells; colloid; vasculature (lumen or wall); and "other" (primarily clear cells).

This procedure was performed on 10 thyroids from each experimental group in Experiment I, and from each experimental group in Experiment II (except for only 3 thyroids in the 640 rads + 40 ppm MMI group). The ten thyroids from each group were selected at random, except for one bias, from the survivors of that group. The bias consisted of rejecting an animal if the thyroid gland of that animal was so overrun with tumor tissue as to make it impossible to measure 10 adjacent fields in a section. It was for this reason that only 3 animals were quantified in the 640 rads + 40 ppm MMI group in Experiment I; the other survivors in this group had insufficient tumor free tissue to permit valid quantitative measurement.

In four animals, the quantification was done on at least 4 sections throughout the gland, in order to verify whether or not quantification in one section of the gland was representative of the entire gland. Student's 't' test revealed no statistically significant differences in any of the variables measured ($p > .05$).

Tissues other than the thyroid were examined for possible metastases if the thyroid had a tumor. The pituitary glands were examined for tumors. In five to ten of the pituitaries from each experimental group, estimation of the number of thyrotrophs was made, using a grading scale of 0 to +3.

5. Statistical Methods

The mean and standard error of the mean were calculated for each parameter, for every group. Two way analysis of variance (Bliss, 1967) was used to determine statistical significance among the various groups for the parameters of change in body weight, thyroid weight, thyroid weight , and the following histologically quantified thyroid body weight gland components: vasculature, connective tissue, colloid, and epithelial tissue.

The general model tested in the two way analysis of variance was of the form $\text{Effect}_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \Sigma_{ijk}$, where Effect_{ijk} is the parameter being tested (weight change, thyroid weight, etc.), i denotes the level of MMI, j denotes the level of radiation, k denotes a specific rat within the (i,j) experimental group, μ represents the overall average value of the parameter being tested, α_i represents the effect of the i th level of MMI, β_j represents the effect of the j th level of radiation, γ_{ij} represents the interaction between the i th level of MMI and j th level of radiation, and Σ_{ijk} are assumed independent and identically distributed random variables, with mean=0 and variance= σ^2 .

Thyroid tumor incidence data were evaluated with χ^2 tests (Grizzle, et al., 1969) using the log model $\ln(1 - P_{ij}) = \mu + \alpha_i + \beta_j + \gamma_{ij}$, where $P_{ij} =$

probability of developing a tumor in the experimental group getting the i th level of MMI and the j th level of radiation, and the other symbols are as previously defined.

IV. RESULTS

A. Experiment I

1. Survival Data

The survival data, fraction of each rat group surviving at 13 months post irradiation in Experiment I, are given in Table 2. No significant trends in survival are evident. This impression is confirmed by analysis of variance (ANOVA) testing for each batch of rats and for all four batches combined, which shows no MMI or radiation effect on survival ($.05 < p$).

2. Change in Body Weight

The group initial mean weights ranged from 216 to 233 grams (Table 3). Changes in body weights of surviving rats at 13 months post irradiation in Experiment I are presented in Table 4 and Figure 2. Evaluating change in weight, rather than percent change in weight, is valid since ANOVA revealed no statistically significant difference among the initial mean weights of the 15 experimental groups ($p = .58$). ANOVA suggests no evidence of interaction between the two experimental treatments (MMI and radiation) on change in body weight ($.05 < p < .10$). The effect of radiation treatment on body weight was to significantly reduce the normal increase in body weight at the higher radiation dose ($p < .005$), (Figure 2). The effect of MMI treatment on body weight was not significant ($.05 < p < .10$).

3. Thyroid Assembly Weight

Thyroid assembly weights (thyroid and attached cut segments of

TABLE 2

Survival of Rats Post Irradiation
Fraction Surviving (%)

MMI ^a (ppm)	Thyroid Radiation Dose (rads)						Combined
	0	80	160	320	640		
	Experiment I (13 months post-irradiation)						
0	17/51 (33)	24/53 (45)	24/47 (51)	15/50 (30)	17/56 (30)	97/257 (38)	
4	18/50 (36)	23/47 (49)	26/45 (58)	10/43 (23)	11/49 (22)	88/234 (38)	
40	<u>20/50 (40)</u>	<u>15/43 (35)</u>	<u>27/50 (54)</u>	<u>21/46 (46)</u>	<u>15/36 (42)</u>	<u>98/225 (44)</u>	
Combined	55/151 (36)	62/143 (43)	77/142 (54)	46/139 (33)	43/141 (30)	283/716 (40)	
	Experiment II (7-1/2 months post-irradiation)						
200	28/35 (80)	22/34 (65)	25/33 (76)	25/33 (76)	25/34 (74)	125/169 (74)	

^a Concentration of methimazole (MMI) in the drinking water in parts per million (ppm)

TABLE 3

Mean Initial Weight of Surviving Rats gm $\pm$ S.D. (number of rats/group) Thyroid Radiation Dose (rads)						
	0	80	160	320	640	Mean
MMI ^a (ppm)	Experiment I (13 months post-irradiation)					
0	229.2 $\pm$ 18.7	223.4 $\pm$ 18.7	228.0 $\pm$ 14.7	228.1 $\pm$ 23.8	228.1 $\pm$ 11.2	226.0 $\pm$ 17.5
4	221.7 $\pm$ 17.1	229.1 $\pm$ 17.8	229.1 $\pm$ 22.6	232.7 $\pm$ 27.2	226.1 $\pm$ 18.9	227.6 $\pm$ 20.3
40	222.5 $\pm$ 18.2	216.0 $\pm$ 15.2	228.3 $\pm$ 18.9	229.1 $\pm$ 13.2	224.6 $\pm$ 18.3	224.9 $\pm$ 17.2
Mean	<u>224.3$\pm$18.0</u>	<u>223.7$\pm$18.0</u>	<u>228.5$\pm$18.9</u>	<u>229.5$\pm$19.9</u>	<u>223.9$\pm$15.8</u>	<u>226.1$\pm$18.3</u>
	Experiment II (7-1/2 months post-irradiation)					
200	249.1 $\pm$ 26.9	243.0 $\pm$ 28.3	251.3 $\pm$ 34.5	250.8 $\pm$ 24.1	236.0 $\pm$ 23.2	246.1 $\pm$ 27.9

^aConcentration of methimazole (MMI) in the drinking water in parts per million (ppm)

TABLE 4

Mean Change in Body Weight of Surviving Rats^a
From Initial Body Weight
gm±S.D.
(number of rats/group)

	Thyroid Radiation Dose (rads)					
	0	80	160	320	640	Mean
MMI ^b (ppm)	Experiment I (13 months post-irradiation)					
0	59.6±35.6 (17)	37.0±34.0 (24)	56.4±49.9 (22)	20.7±46.0 (15)	35.7±40.4 (17)	42.7±42.9 (95)
4	49.3±22.8 (18)	41.6±40.4 (23)	62.2±37.7 (26)	42.1±34.8 (10)	19.5±31.1 (11)	46.5±36.5 (88)
40	63.3±31.3 (20)	56.9±33.0 (15)	55.6±25.0 (27)	36.6±30.4 (21)	45.1±31.2 (14)	51.5±30.7 (99)
Mean	57.6±30.4 (55)	43.5±36.6 (62)	58.1±37.6 (75)	32.8±37.0 (46)	34.6±35.8 (42)	47.0±37.0 (280)
	Experiment II (7-1/2 months post-irradiation)					
200	-20.9±24.0 (28)	-12.5±26.1 (22)	-11.5±31.5 (25)	-16.7±16.2 (23)	- 1.1±16.5 (25)	-12.7±24.2 (123)

^aThe number of animals is not the same as the number sacrificed, since some weights were not recorded.

^bConcentration of methimazole (MMI) in the drinking water in parts per million (ppm)

trachea and esophagus) for the 15 groups in Experiment I are shown in Table 5 and Figure 3. Two way ANOVA shows no evidence of interaction between the two experimental treatments (MMI and radiation) on mean thyroid assembly weight ($.10 < p$). There is evidence that radiation and MMI separately altered thyroid assembly weight ($p < .025$, $p < .005$ respectively). However, since in this analysis the error variances were not homosedastic, and the error distribution was skewed, implying that the conditions for the test were not perfectly met, the validity of the significance of the radiation effect is questionable. Figure 3 indicates that radiation had no effect or slightly decreased mean thyroid assembly weight for the 0 ppm MMI groups, had little effect on the 4 ppm MMI mean thyroid assembly weights, and increased the 40 ppm MMI mean thyroid assembly weights. The p-value of the MMI effect is so small ($p < .005$) that even though the variances are not equal among all the groups, it is likely that the effect found (increasing thyroid assembly weight with increasing MMI treatment) is significant.

Some of the increase in thyroid assembly weight in the 40 ppm MMI groups, as opposed to the 0 and 4 ppm groups, is due to the presence of large thyroid tumors. The variation in tumor size is probably the source of the large increase in variance of the thyroid assembly weights of the 40 ppm MMI groups as opposed to the 0 and 4 ppm groups.

The ratio of thyroid assembly weight to body weight was examined to ascertain whether this statistic would reveal any evidence of

TABLE 5

Mean Thyroid Assembly Weight^a of Surviving Rats^b
 mg±S.D.
 (number of rats/group)

Thyroid Radiation Dose (rads)						
	0	80	160	320	640	Mean
MMI ^C (ppm)	Experiment I (13 months post-irradiation)					
0	174.5±18.2 (11)	158.9±22.9 (24)	164.3±21.8 (23)	146.5±26.3 (13)	155.9±21.8 (17)	159.9±23.0
4	165.8±15.2 (17)	169.5±20.2 (21)	178.9±22.7 (25)	171.5±23.2 (10)	159.0±15.6 (11)	170.4±20.6
40	231.8±65.3 (15)	240.0±41.3 (14)	304.5±154 (27)	261.6±73.2 (20)	274.7±191.0 (15)	268.3±124
Mean	191.1±50.1	181.9±42.5	219.7±113.1	205.9±74.8	199.1±126.2	200.9±89.7
	Experiment II (7-1/2 months post-irradiation)					
200	280.0±65.8 (28)	319.9±35.5 (22)	279.4±71.4 (24)	277.3±77.1 (24)	209.8±38.9 (24)	272.8±76.3

^aThyroid Assembly consists of thyroid, with attached cut segments of trachea and esophagus

^bThe number of animals is not the same as the number sacrificed, since some weights were not recorded.

^cConcentration of methimazole (MMI) in the drinking water in parts per million (ppm)

FIGURE 3
MEAN THYROID ASSEMBLY WEIGHT
OF SURVIVING RATS
(mg)

interaction between MMI and radiation (Table 6, Figure 4). As in the ANOVA of the mean thyroid assembly weight, the variances in the ANOVA of the ratio of the weights were not equal. In particular, the variance of the mean ratio in the 160 rads + 40 ppm MMI group was nearly twice as large as the next largest variance (52.7 vs. 28.2 mg/100 gm). Furthermore, all the variances of the ratios of the 40 ppm MMI groups were much larger than the variances of the ratios of the 0 and 4 ppm MMI groups. Since the condition of equal variances was not met, the ANOVA finding of interaction between radiation and MMI ($.025 < p < .05$) may not be significant. No effect of radiation alone on the ratio was found ($.05 < p$). It was found that MMI treatment significantly ($p < .005$) increased the size of the thyroid assembly weight to body weight ratio.

4. Histology of Non-Tumorous Thyroid Tissue

a. Qualitative histology

(1) Control rats (non-irradiated, non-treated)

The control thyroid glands showed the previously reported variation in follicle size, with larger follicles at the periphery of the gland and smaller follicles toward the center of the gland (Plate I). The follicular epithelial cells were squamous to low cuboidal. Vacuoles were occasionally seen in the colloid. Interfollicular fibrillar connective tissue appeared throughout the gland, and bands of connective tissue divided the gland into lobes.

TABLE 6
Mean Ratio Thyroid Assembly Weight^a of Surviving Rats^b
Body Weight
mg/100 gm±S.D.
(number of rats/group)

		Thyroid Radiation Dose (rads)					Mean
		0	80	160	320	640	
MMI ^c (ppm)	Experiment I (13 months post-irradiation)						
0	60.9±6.4 (11)	61.6±7.8 (24)	59.1±9.7 (23)	57.5±11.0 (13)	61.6±7.6 (16)	60.2±8.6 (87)	
4	61.3±8.3 (17)	63.9±11.3 (21)	61.5±9.1 (25)	62.6±6.3 (10)	65.2±7.2 (11)	62.7±9.0 (84)	
40	82.1±28.2 (15)	88.3±15.0 (14)	107.5±52.7 (27)	100.3±26.9 (20)	85.5±19.3 (14)	95.2±35.8 (90)	
Mean	68.4±20.1	68.8±15.5	77.3±39.4	78.6±28.2	70.7±16.5	73.1±27.4	
		Experiment II (7-1/2 months post-irradiation)					
200	123.7±31.2 (29)	138.7±35.2 (22)	116.5±28.3 (24)	118.8±34.2 (24)	89.1±14.3 (24)	117.3±33.1 (123)	

^aThyroid Assembly consists of the thyroid with attached cut segments of trachea and esophagus

^bThe number of animals is not the same as the number sacrificed, since some weights were not recorded.

^cConcentration of methimazole in the drinking water in parts per million (ppm)

PLATE I

Histologic section of thyroid from a nontreated control rat sacrificed 13 months after the start of Experiment I, showing heterogeneity of follicle size with the typical pattern of larger follicles at the periphery of the gland. Hematoxylin and Eosin, 41X.

PLATE II

Histologic section of thyroid from a rat sacrificed 7-1/2 months after the beginning of treatment (0 rad + 200 ppm MMI), showing a vasculitis-like lesion surrounding the two major vessels in the field. Hematoxylin and Eosin, 103X.

Many of the thyroid glands examined, regardless of experimental group, showed a clump of flattened epidermoid cells which formed concentric circles. These structures had the appearance of follicles into which epithelial cells had been sloughed. The formations resembled psammoma bodies as described in human thyroid pathology. Psammoma bodies in the human thyroid are generally taken as being diagnostic of thyroid carcinoma. This, however, was not true for the rat thyroids of this experiment. No correlation was seen between the epidermoid bodies and benign or malignant tumor incidence as seen in semi-serial sections of the thyroid. Similar structures have been seen in animals in other experiments. Marks et al. (1957) reported seeing, in normal sheep thyroid glands, "islands of stratified squamous epithelium which are frequently cystic, resembling small epidermoid cysts of human skin".

A vascular lesion resembling polyarteritis consisting of inflammation of and fibrosis about small arteries in and around the thyroid was seen in approximately 5% of the animals (Plate II). The incidence seemed to decrease with increasing radiation dose. No variation in incidence with MMI treatment was noted.

Follicular epithelial cells consisting almost entirely of a single large clear vacuole were seen in some thyroids. These cells typically appeared in a cluster. The vacuole frequently was so large as to push the nucleus of the cell toward the cellular membrane, giving the cell a signet ring appearance. The incidence of thyroids with these cells was strongly related to MMI treatment,

being much higher in the 40 ppm MMI treated rats. Axelrad and Leblond (1955) reported seeing similar cells in rats fed an iodine deficient diet for 25 months.

(2) 0 and 4 ppm MMI experimental groups

At the same radiation dose levels, there were no observable differences among the thyroids of rats in the 0 and 4 ppm MMI groups. That is, the 4 ppm MMI treatment caused no noticeable change in the thyroid at the light microscope level. Radiation effects on the thyroid glands were detected, but only at the 640 rad level. The thyroids at this dose level contained follicles typically smaller than those in the thyroid glands of the control animals. The colloid frequently had a clumped or inspissated appearance. The follicular epithelial cells exhibited more cytoplasmic vacuoles than did similar cells in the control non-irradiated thyroid glands. Hyperchromatism of follicular cell nuclei was more prevalent than in non-irradiated control glands. Occasionally the vasculature of the glands which received 640 rads showed changes as compared to the vasculature of the glands from non-irradiated control rats. These changes consisted primarily of decreased vessel lumen diameter and increased connective tissue about the vessel. Some of the thyroids which received 640 rads of radiation showed an increased amount of interstitial connective tissue throughout the gland. This appeared both as thickening of the connective tissue septa separating the thyroid lobules, and as increased numbers of such septa.

(3) 40 ppm MMI experimental groups

The difference in histologic appearance between the thyroids of animals in the 40 ppm MMI experimental groups (Plate III) and the thyroids of the 0 and 4 ppm MMI experimental groups (Plate I) was marked. The amount of colloid in an average follicle was greatly diminished. The height of the follicular epithelial cells was increased, so that the median follicular cell was cuboidal to columnar in morphology. The cross-sectional size of the gland was significantly greater than in the 0 or 4 ppm MMI treated rats. The follicular colloid of the 40 ppm MMI rats did not stain as intensely as did the colloid of the 0 and 4 ppm MMI rats. The 640 rad level of radiation caused changes similar to those seen in the 0 and 4 ppm MMI 640 rad groups: clumped colloid and increased connective tissue. No effects of radiation were seen at the other radiation dose levels used.

b. Quantitative histology

The results of the quantitative histologic studies of rat thyroids are given in Tables 7-10 and Figures 5-8. The tissue components quantified in terms of relative area of non-tumorous thyroid occupied were: 1) connective tissue; 2) colloid; 3) follicular epithelial tissue; and 4) vasculature.

Two way ANOVA of the data from experiment I indicates an interaction between MMI and radiation on the connective tissue component of the thyroid ($p < .025$, Table 7, Figure 5). The ANOVA determination of interaction appears to primarily come from the

PLATE III

Histologic section of thyroid from a rat sacrificed 13 months after the beginning of treatment (0 rad + 40 ppm MMI), showing decreased follicular size, and increased thyroid epithelial area in response to MMI induced TSH stimulation. Compare with the appearance of the nontreated control gland in Plate I. Hematoxylin and Eosin, 41X.

PLATE IV

Histologic section of thyroid from a rat sacrificed 13 months after the beginning of treatment (160 rads + 40 ppm MMI), showing a thyroid adenoma. Hematoxylin and Eosin, 165X.

TABLE 7

Mean Relative Area (%) of Thyroid Occupied by
Connective Tissue
(%±S.D.)

Thyroid Radiation Dose (rads)						
	0	80	160	320	640	Mean
MMI ^a (ppm)	Experiment I (13 months post-irradiation)					
0	12.6±7.8	15.5±2.5	15.6±2.5	18.1±3.3	17.4±2.0	15.8±3.2
4	16.3±3.0	14.2±2.2	15.8±2.5	15.6±2.5	20.3±4.0	16.4±3.6
40	15.7±2.6	15.4±3.3	14.7±3.4	17.3±2.3	20.1±3.0 ^b	16.1±3.2
Mean	14.9±3.2	15.1±2.7	15.7±2.7	17.0±3.1	19.0±3.3	16.1±3.3
Experiment II (7-1/2 months post-irradiation)						
200	16.6±3.5	17.0±2.8	18.2±3.7	18.8±3.8	21.7±3.8	18.4±3.9

^aConcentration of methimazole in the drinking water in parts per million (ppm)

^bThis value based on 3 rats, all others based on 10 or 11 rats

FIGURE 5
MEAN RELATIVE AREA (%) OF THYROID OCCUPIED BY
CONNECTIVE TISSUE

TABLE 8

Mean Relative Area (%) of Thyroid Occupied by
Colloid
(%±S.D.)

Thyroid Radiation Dose (rads)						
	0	80	160	320	640	Mean
MMI ^a (ppm)	Experiment I (13 months post-irradiation)					
	0	36.5±8.9	32.9±7.6	35.4±8.4	27.9±6.0	30.9±4.9
4	25.8±6.2	29.9±4.3	32.4±9.9	28.8±6.9	22.1±5.1	27.7±7.3
40	<u>15.5±4.2</u>	<u>18.5±7.7</u>	<u>17.1±5.9</u>	<u>15.0±7.4</u>	<u>8.6±5.3^b</u>	<u>16.0±6.6</u>
	Experiment II (7-1/2 months post-irradiation)					
200	12.1±6.0	10.9±5.4	10.4±3.3	8.6±4.7	8.2±4.1	10.1±4.9

^aConcentration of methimazole in the drinking water in parts per million (ppm)

^bThis value based on 3 rats, all others are based on 10 or 11 rats.

FIGURE 6
MEAN RELATIVE AREA (%) OF THYROID OCCUPIED BY COLLOID

FIGURE 7
MEAN RELATIVE AREA (%) OF THYROID OCCUPIED BY
EPITHELIAL TISSUE

TABLE 10

Mean Relative Area (%) of Thyroid Occupied by
Vasculature
(%±S.D.)

Thyroid Radiation Dose (rads)						
	0	80	160	320	640	Mean
MMI ^a (ppm)	Experiment I (13 months post-irradiation)					
0	9.5±2.0	8.6±2.9	10.4±2.3	9.4±3.8	9.4±2.9	9.5±2.8
4	10.3±1.8	11.6±1.6	8.6±3.2	9.5±2.6	8.5±2.1	9.8±2.5
40	<u>12.1±3.7</u>	<u>11.1±4.6</u>	<u>10.3±5.4</u>	<u>10.7±3.5</u>	<u>9.9±3.4^b</u>	<u>11.0±4.2</u>
Mean	10.6±2.8	10.4±3.4	9.9±3.7	9.9±3.3	9.1±2.6	10.0±3.2
	Experiment II (7-1/2 months post-irradiation)					
200	11.5±3.2	13.5±4.1	11.7±2.9	13.2±3.6	10.5±3.0	12.0±3.4

^aConcentration of methimazole in the drinking water in parts per million (ppm)

^bThis value based on 3 rats, all others based on 10 or 11 rats

FIGURE 8
MEAN RELATIVE AREA (%) OF THYROID OCCUPIED BY VASCULATURE

fact that at the 0 and 640 rads of radiation levels, the 0 ppm MMI groups had less connective tissue than did the 4 and 40 ppm MMI groups, while at the 80, 160, and 320 rads of radiation treatment levels the 0 ppm MMI groups had amounts of connective tissue equal to or greater than the 4 and 40 ppm MMI groups.

Thyroid irradiation alone very significantly increased the relative amount of connective tissue component in the glands examined ($p < .005$). The increase was largest at the 640 rad level, noticeable at the 320 rad level, and questionable at the 160 and 80 rad level. This finding is consistent with the hypothesis that radiation damage in a tissue is repaired via connective tissue proliferation. No consistent effect of MMI alone on connective tissue was found at the levels of MMI used ($.25 < p$).

The other three tissue components examined, i.e., colloid, vasculature, and epithelial tissue, did not show evidence of interaction between radiation and MMI treatments ($.10, .25, .50 < p$ respectively). All three, however, showed very significant effects of MMI treatment ($p < .005$). The relative amounts of follicular epithelial tissue and of vasculature increased with increasing MMI level, while the relative amount of colloid decreased with increasing MMI level.

The effects of thyroid irradiation alone on relative amounts of colloid, follicular epithelium, and vasculature, while not as obvious as, and more complex than, those of MMI were indicated to be statistically significant by ANOVA ($p < .005, .025, .025$ respectively).

The relative amount of colloid increased over that in the non-irradiated control in the 4 and 40 ppm MMI groups at the lower (80 and 160 rads) radiation dose levels. At the higher radiation doses (320 and 640 rads), the relative amount of colloid in the 4 and 40 ppm MMI groups was approximately equal to or less than the amount in the corresponding non-irradiated control group. In the 0 ppm MMI groups the amounts of colloid generally decreased with increasing radiation dose.

The effects of irradiation on the relative amount of epithelial tissue were slight. In general, at low doses of radiation (80 and 160 rads) the relative amount of epithelial tissue decreased, while at higher doses of radiation the relative amount of epithelial tissue increased or remained the same.

The relative amount of vasculature generally decreased with increasing radiation dose for the 4 and 40 ppm MMI groups. Whether radiation had any significant effect on the vasculature in the 0 ppm MMI groups is questionable, although if there was no effect in the 0 ppm MMI groups, and some effect in the 4 and 40 ppm MMI groups, the interaction term in the two-way ANOVA would be expected to be found significant, which it was not.

5. Thyroid Tumors

a. Description and definitions

The following thyroid tumor classification scheme was used for this study:

- A. Follicular Adenoma
- B. Papillary Adenoma
- C. Mixed Follicular/Papillary Adenoma
- D. Medullary Tumor
- E. Carcinoma

The benign follicular, papillary and mixed adenomas generally had well defined capsules. The adjacent glandular tissue sometimes exhibited compression. The adenomas appeared in a wide range of sizes, from approximately 50 μm to 1000 μm or more in diameter. Colloid in the follicles in these tumors exhibited staining intensity ranging from pale to dark. The follicular epithelial cells generally stained moderately to darkly. Degenerative changes, including hemorrhage and fibrosis, were occasionally seen in these tumors.

The follicles in follicular adenomas frequently resembled those in normal thyroid tissue. The range of follicle diameter in these tumors was greater than in the non-tumorous gland, ranging from a few to hundreds of micrometers (Plate IV, V). The papillary adenomas appeared similar to the follicular adenomas, except that the papillary form had papillae projecting into the lumens of the follicles. The mixed follicular/papillary adenomas exhibited both types of characteristic architectures.

It was difficult to classify some of the adenomas since the degree of difference among follicular, papillary and mixed follicular/papillary forms was not large. Therefore, the follicular adenomas, papillary adenomas, and mixed follicular/papillary

PLATE V

Histologic section of thyroid from a rat sacrificed 13 months after the beginning of treatment (160 rads + 40 ppm MMI), showing 2 moderate sized adenomas. Hematoxylin and Eosin, 41X.

PLATE VI

Histologic section of thyroid from a rat sacrificed 13 months after the beginning of treatment (160 rads + 40 ppm MMI), showing in the center a cellular medullary tumor enveloping several follicles. Hematoxylin and Eosin, 165X.

adenomas were combined to form one class of adenomas for the data analysis. This method of classification has been used by Doniach (1975).

Thyroid medullary tumors occur naturally in the rat (Lindsay et al. 1968a). As opposed to the non-medullary tumors (follicular and papillary adenomas), which arise from follicular epithelial cells, medullary tumors are believed to arise from the clear cells of the thyroid gland. The medullary tumor is highly cellular, with pale staining cells and no colloid (Plate VI). Due to these characteristics, it is possible to differentiate medullary tumors from non-medullary tumors.

Malignant tumors were classified as such using the criterion of invasion of thyroid (Plate VII) or other tissue as the strongest indicator of malignancy. If invasion was questionable, then the criteria of 1) cellular pleomorphism, 2) cellular anaplasia, and 3) high mitotic activity were applied. If all of these three criteria were well satisfied, the tumor was classified as malignant. If one of these criteria was absent or questionable, the tumor was classified as "undecided". If more than one of these three criteria were absent or questionable the tumor was classified benign.

No metastases were found in any of the non-thyroid tissues examined. Transgression of the thyroid capsule by thyroid tumors was not observed. However, thyroid tumor cells were seen invading venous blood vessels in some cases and growing inside blood vessels in two thyroids (Plate VIII). The presence of the thyroid

PLATE VII

Histologic section of thyroid from a rat sacrificed 13 months after the beginning of treatment (320 rads + 40 ppm MMI), showing a thyroid carcinoma exhibiting invasion toward the center and right. Hematoxylin and Eosin, 41X.

PLATE VIII

Histologic section of thyroid from a rat sacrificed 13 months after the beginning of treatment (320 rads + 40 ppm MMI), showing carcinoma cells in a thyroid blood vessel. Hematoxylin and Eosin, 165X.

metastases might have been found if more detailed examination of the non-thyroidal tissues had been undertaken, or if more time had been allowed to pass before the animals were sacrificed.

b. Incidences of rats with thyroid tumors

The incidences of rats with thyroid tumors are given in Table 11 and Figure 9. Of the 283 rats sacrificed at 13 months post-irradiation in Experiment I, 121 (43%) had tumorous thyroid glands. The numbers of rats with tumorous thyroids by tumor types were: medullary tumors only, 22 (see Figure 10); non-medullary tumors only, 91 (see Figure 11); rats with both medullary and non-medullary tumors, 8 (see Figure 12). Of the 91 rats having thyroid tumors which were non-medullary types only, 13 had malignant non-medullary tumors (see Figure 13), while 78 had benign non-medullary tumors.

The 0 and 4 ppm MMI groups had approximately the same incidence of rats with tumors, 21 and 17%, respectively, while the 40 ppm MMI groups had a significantly higher incidence of rats with tumors (88%, $p < .001$). The incidences of rats with thyroid tumors in both the 0 and 4 ppm MMI experimental groups show a pattern of increasing incidence with increasing radiation dose up to 320 rads, with no further increase, or a slight decline from the peak levels with further increase in radiation dose to 640 rads. A roughly similar trend is evident in the 40 ppm MMI groups, with a 65% incidence at 0 rads and a rise to 96% at 160 rads, with incidence levels of 91% and 100% at 320 rads and 640 rads, respectively.

TABLE 11

INCIDENCE OF RATS WITH BENIGN (B) AND MALIGNANT (M) THYROID TUMORS
EXPERIMENT I (13 MONTHS POST-IRRADIATION)
(NUMBER OF RATS WITH THYROID TUMOR) %

Experimental Group	Number of Rats	Tumors Of Any Type				Medullary Tumors Only		Non-Medullary Tumors Only			Rats With Medullary + Non-Medullary Tumors	
		(B)	(M)	Sum		(B)	(M)	(B)	(M)	Sum	(B)	(M)
0 ppm MMI ^a +												
0 rads	17	(0) 0	(1) 6	(1) 6		- (1) 6		0 0	-		-	-
80 rads	24	(1) 4	(4) 17	(5) 21		- (4) 17		(1) 4	-		-	-
160 rads	24	(2) 8	(4) 17	(6) 25		- (4) 17		(2) 8	-		-	-
320 rads	15	(1) 7	(3) 20	(4) 27		- (3) 20		(1) 7	-		-	-
640 rads	17	(1) 6	(3) 19	(4) 25		- (2) 12		(1) 6	(1) 6	(2) 12	-	-
Mean	97	(5) 5	(15) 15	(20) 21		(14) 14		(5) 5	(1) 6	(6) 6		
4 ppm MMI +												
0 rads	18	(0) 0	(0) 0	(0) 0		- (0) 0		(0) 0	-		-	-
80 rads	23	(2) 9	(2) 9	(4) 18		- (2) 9		(2) 9	-		-	-
160 rads	26	(2) 8	(4) 16	(6) 24		- (3) 12		(2) 8	-		-	(1) 4
320 rads	10	(1) 10	(2) 20	(3) 30		- (2) 20		(1) 10	-		-	-
640 rads	11	(2) 18	(0) 0	(2) 18		- (0) 0		(2) 18	-		-	-
Mean	88	(7) 8	(8) 9	(15) 19		(7) 8		(7) 8	-	(7) 8	(1) 1	
40 ppm MMI +												
0 rads	20	(11) 55	(2) 10	(13) 65		- (0) 0		(11) 55	(1) 5	(12) 60	-	(1) 5
80 rads	15	(12) 80	(1) 7	(13) 87		- (0) 0		(12) 80	(1) 7	(13) 87	-	(0) 0
160 rads	27	(18) 67	(8) 30	(26) 96		- (1) 4		(18) 67	(4) 15	(22) 82	-	(3) 11
320 rads	21	(15) 71	(4) 18	(19) 91		- (0) 0		(15) 71	(3) 14	(18) 86	-	(1) 15
640 rads ^b	15	(10) 67	(5) 33	(15) 100		- (0) 0		(10) 67	(3) 20	(13) 87	-	(2) 13
Mean	98	(66) 67	(20) 20	(86) 87		(1) 1		(66) 67	(12) 12	(88) 80	-	(7) 8
Grand Mean	283	(78) 28	(43) 15	(121) 43		(22) 8		(78) 28	(13) 5	(91) 32		(8) 3

^aConcentration of methimazole in the drinking water in parts per million (ppm)

^bOne rat had a thyroid tumor of questionable malignancy, scored as benign

FIGURE 9
INCIDENCE OF RATS WITH THYROID TUMORS
EXPERIMENT 1, (13 MONTHS POST-IRRADIATION)

FIGURE 10
INCIDENCE OF RATS WITH MEDULLARY THYROID TUMORS ONLY
EXPERIMENT I (13 MONTHS POST-IRRADIATIONS)

FIGURE 11
INCIDENCE OF RATS WITH NON-MEDULLARY THYROID TUMORS ONLY
EXPERIMENT I (13 MONTHS POST-IRRADIATIONS)

FIGURE 12
INCIDENCE OF RATS WITH BOTH MEDULLARY AND
NON-MEDULLARY THYROID TUMORS
EXPERIMENT I (13 MONTHS POST-IRRADIATION)

FIGURE 13
INCIDENCE OF RATS WITH MALIGNANT NON-MEDULLARY TUMORS ONLY
EXPERIMENT I, (13 MONTHS POST-IRRADIATION)

With one exception, the malignant tumors in the 0 to 4 ppm MMI groups were of the medullary or naturally occurring tumor type (Table 11). The one exception was a malignant non-medullary tumor in the 640 rads + 0 MMI group (Figure 13). The incidences of rats with medullary tumors only were 14% in the 0 ppm MMI groups and 8% in the 4 ppm MMI group. These incidences are not statistically different (χ^2 , $.10 < p$). In both the 0 and 4 ppm MMI groups, the incidence of rats with medullary tumors only (Figure 10) increased with increasing radiation dose up to 320 rads and then at 640 rads declined from the peak considerably (0 ppm MMI) or returned to zero (4 ppm MMI). However, with the number of animals used in this experiment, and the incidences of medullary tumors observed, the trend is not statistically significant according to the χ^2 test ($.05 < p$). The incidences of rats with non-medullary tumors only (Figure 11), comparing the 0 and 4 ppm MMI groups, were not statistically different (5% vs. 8%, χ^2 , $.20 < p$). For both MMI levels the incidence was dependent on radiation dose and increased, to a small degree, at the highest radiation dose (640 rads).

The 40 ppm MMI groups had smaller incidences of rats with medullary tumors only than did the 0 and 4 ppm MMI groups (1% vs. 14% and 8% respectively; χ^2 , $p < .025$). However, the thyroid of 8% of the rats in the 40 ppm MMI groups contained both a medullary and non-medullary tumor, while only 1% of the 4 ppm MMI rats and none of the 0 ppm MMI rats had both thyroidal medullary and non-medullary tumors. The incidence of rats in the 40 ppm MMI groups having

non-medullary thyroid tumors only was 78%. The 78% incidence was made up of a benign tumor incidence of 66% and a malignant tumor incidence of 12%. The mean incidence of rats with benign non-medullary tumors only, was greater in the four irradiated groups (55/78, 71%) than in the non-irradiated group (11/20, 55%), although not significantly so (χ^2 , $.10 < p$). The incidence of rats with malignant non-medullary tumors only tended to increase with increasing radiation dose (Figure 13) although for the number of animals used and the incidences observed, the trend is not statistically significant (χ^2 , $.05 < p$). The incidence of rats in the 40 ppm MMI experimental groups having thyroids containing both medullary and non-medullary thyroid tumors showed no effect of thyroid radiation.

c. Excess incidence of rats with thyroid tumors

Excess tumor incidence is defined as the difference in tumor incidence between an experimental group and its corresponding control group. The excess incidence of rats with thyroid tumors was calculated for rats with: 1) medullary thyroid tumors only, 2) non-medullary thyroid adenomas only, 3) non-medullary thyroid carcinomas only, 4) both medullary and non-medullary thyroid tumors, and 5) any thyroid tumor. For each experimental group (x rads + Y ppm MMI) and thyroid tumor type the excess tumor incidence was calculated using as the control group: 1) the non-treated group 0 rads + 0 ppm MMI; 2) the appropriate radiation-only group (X rads + 0 ppm MMI); and 3) the appropriate MMI-only group (0 rads + Y MMI).

The non-treated group, 0 rads + 0 ppm MMI, was used to calculate the excess incidence of rats with tumors in an experimental group due to treatment with irradiation only, MMI only, or irradiation and MMI combined. In addition, for each experimental group, the excess incidence of animals with thyroid tumors due to MMI treatment was determined by using as control the experimental group which received the same level of radiation and no MMI. Similarly, the excess incidence of animals with thyroid tumors as a result of irradiation was calculated for an experimental group by using as control the group which received the same level of MMI treatment and no radiation.

The data on excess incidence of rats with thyroid tumors are summarized in Tables 12 and 13. These two tables present the same data, but organized differently.

The following observations on excess incidence attributable to MMI treatment are made by comparing the incidence in radiation + MMI groups with the appropriate radiation-only control groups (Table 12):

- 1) the incidence of rats with medullary tumors only generally decreased with increasing MMI dosage; 2) the incidence of rats with non-medullary adenomas only was increased greatly by MMI treatment at the 40 ppm MMI level; 3) the incidence of rats with non-medullary carcinomas only was not affected by MMI treatment at the 4 ppm MMI level, but was increased by the 40 ppm MMI dosage; 4) the incidence of rats having both medullary and non-medullary thyroid tumors was increased slightly by the 40 ppm MMI treatment at all radiation levels except the 80 rad level; and 5) the incidence of rats with

TABLE 12

EXCESS INCIDENCE OF RATS WITH THYROID TUMORS - EXPERIMENT I
(GROUPED BY THYROID RADIATION DOSE LEVELS)

Group	Types Of Tumors In Thyroid														
	Medullary Only			Non Medullary Adenoma			Non Medullary Carcinoma			Medullary + Non Medullary			Any Type		
	Control			Control			Control			Control			Control		
	1 ^a	2 ^b	3 ^c	1 ^a	2 ^b	3 ^c	1 ^a	2 ^b	3 ^c	1 ^a	2 ^b	3 ^c	1 ^a	2 ^b	3 ^c
0 rads															
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-6	-6	-	0	0	-	0	0	-	0	0	-	-	-6	-
40	-6	-6	-	55	55	-	5	5	-	5	5	-	59	59	-
Mean	-6	-6	-	28	27	-	2	2	-	2	2	-	26	26	-
80 rads															
0	11	-	11	4	-	4	0	-	0	0	-	0	15	-	15
4	3	-8	9	9	5	9	0	0	0	0	0	0	12	-3	18
40	-6	-17	0	80	76	25	7	7	2	0	0	-5	81	66	22
Mean	3	-12	7	31	40	13	2	4	1	0	0	-2	36	32	18
160 rads															
0	11	-	11	8	-	8	0	-	0	0	-	0	19	-	19
4	6	-5	12	8	0	8	0	0	0	4	4	4	18	-1	24
40	-2	-13	4	67	59	12	15	15	10	11	11	6	90	71	31
Mean	-5	-9	9	28	30	9	5	8	3	5	8	3	42	35	25
320 rads															
0	14	-	14	7	-	7	0	-	0	0	-	0	21	-	21
4	14	0	20	10	3	10	0	0	0	0	0	0	24	3	30
40	-6	-20	0	71	64	16	14	14	9	5	5	0	84	63	25
Mean	7	-10	11	29	34	11	5	7	3	2	2	0	43	34	26
640 rads															
0	6	-	6	6	-	6	0	-	0	0	-	0	12	-	12
4	-6	-12	0	18	12	18	0	0	0	0	0	0	12	0	18
40	-6	-12	0	67	61	12	20	20	15	13	13	8	94	82	35
Mean	-2	-12	2	30	36	12	7	10	5	4	6	4	39	41	22
Grand Mean	1	-10	7	29	33	11	4	6	3	3	4	1	37	34	23

^a0 rads + 0 MMI as control^bEquivalent radiation + 0 MMI as control^c0 rads + equivalent MMI as control

TABLE 13

EXCESS INCIDENCES OF RATS WITH THYROID TUMORS - EXPERIMENT I
(GROUPED BY MMI DOSAGE LEVELS)

Group	Types Of Tumors In Thyroid											
	Medullary Only			Non Medullary Adenoma			Non Medullary Carcinoma			Medullary + Non Medullary		
	Control	1 ^a	2 ^b	3 ^c	Control	1 ^a	2 ^b	3 ^c	Control	1 ^a	2 ^b	3 ^c
0 ppm MMI												
0 rads	-	-	-	-	-	-	-	-	-	-	-	-
80 rads	11	-	11	4	-	4	0	-	0	0	-	0
160 rads	11	-	11	8	-	8	0	-	0	0	-	0
320 rads	14	-	14	7	-	7	0	-	0	0	-	0
640 rads	6	-	6	6	-	6	6	-	6	0	-	0
Mean	11	-	11	6	-	6	1	-	1	0	-	0
4 ppm MMI												
0 rads	-6	-6	-	0	0	-	0	0	-	0	0	0
80 rads	3	-8	9	9	5	9	0	0	0	0	0	0
160 rads	6	-5	12	8	0	8	0	0	0	4	4	4
320 rads	14	0	20	10	3	10	0	0	0	0	0	0
640 rads	-6	-12	0	18	12	18	0	0	0	0	0	0
Mean	2	-6	8	9	4	8	0	0	0	1	1	1
40 ppm MMI												
0 rads	-6	-6	-	55	55	-	5	5	-	5	5	-
80 rads	-6	-17	0	80	76	25	7	7	2	0	0	-5
160 rads	-2	-13	4	67	59	12	15	15	10	11	11	6
320 rads	-6	-20	0	71	64	16	14	14	9	5	5	0
640 rads	-6	-12	0	67	61	12	20	20	15	13	13	8
Mean	-5	-14	1	68	63	16	12	12	9	7	7	2
Grand Mean	2	-10	7	28	34	10	4	6	3			

^a0 rads + 0 ppm MMI as control^bEquivalent radiation + 0 MMI as control^c0 rads + equivalent MMI as control

any type of thyroid tumor in the 4 ppm MMI groups was not significantly different from the incidence of rats with any type of thyroid tumor in the 0 ppm MMI groups, while the effect of the 40 ppm MMI treatment was to significantly increase the incidence of rats with any type of tumor. It should be noted that most of the increase in rats with tumors in the "any type of tumor" category reflects the increase in the non-medullary adenoma classification.

The excess incidences of rats with thyroid tumors which are attributable to radiation treatment are found by comparing the incidences of rats with thyroid tumors in radiation + MMI groups with the appropriate 0 radiation + MMI control group. As is reflected in Table 12 control 3, all levels of radiation used increased the incidence of rats with tumorous thyroids in all categories of thyroid tumors considered except the category of thyroids with both medullary and non-medullary tumors, where the 80 rad radiation dose reduced the incidence by 2%, and the 320 rad radiation dose which did not alter the incidence. It must be pointed out that many of the increases are small and not statistically significant. It is interesting to note that considering all of the rats with thyroid tumors together as shown in Table 12, an 18% excess in the incidence of tumorous thyroids due to 80 rads of radiation, as compared to the non-irradiated groups, is found, while the doses of 160, 320 or 640 rads show relatively constant excess incidences of thyroid tumors of 25, 26, and 22% respectively.

d. Proportion of thyroid mass occupied by tumor tissue

The proportion or fraction of the thyroid gland mass occupied by tumor tissue, as observed microscopically, was graded on a 0 to +4 scale, as described in the Methods and Materials section. 0 = no tumor; +1 = tumor tissue occupying less than 5% of the thyroid gland mass; +2 = tumor occupying between 5% and 20% of the thyroid mass; +3 = tumor occupying between 20% and 50% of the thyroid mass; and +4 = tumor occupying more than 50% of the thyroid mass. From the method of grading used (proportion of thyroid occupied) it is apparent that three factors influenced the outcome of the grading: 1) the size of the thyroid gland (the larger the gland, the smaller the fraction of the gland occupied by any given tumor); 2) the size of the tumor(s) in the gland; and 3) the number of tumors present (the more tumors of a given size in a gland, the larger the fraction of the gland occupied by tumorous tissue). Thus, the grades indicate only the "relative tumor tissue volume". The results of the grading procedure are shown in Table 14.

Figures 14-16 represent graphically some of the data in Table 14, that is, the percentage of each experimental group which possesses thyroid tumors which are grades from +1 to +4 in terms of the proportion of thyroid gland mass occupied by tumor tissue. Figure 14 shows that the incidence of tumorous thyroids with tumor proportion grades 1 and 2 decreases, and the incidence of tumorous thyroids with grades 3 and 4 increases, with increasing radiation dose in the 40 ppm MMI experimental groups. This is shown in a slightly different

TABLE 14

PERCENT OF RAT THYROID HAVING VARIOUS PROPORTIONS (GRADES) OF THE THYROID GLAND MASS OCCUPIED BY TUMOR TISSUE

		Type of Tumor in Thyroid																Mean Thyroid Grade					
		No Tumor	Medullary Only				Benign Non Medullary Only				Malignant Non Medullary Only				Medullary + Non Medullary				Any Types				
			Grade				Grade				Grade				Grade				Grade				
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
Experiment I (13 Months Post-Irradiation)																							
MMI (ppm)	Radiation (rads)																						
	0	94	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0.06
	80	79	13	4	0	0	4	0	0	0	0	0	0	0	0	0	0	0	17	4	0	0	0.25
	160	75	8	8	0	0	8	0	0	0	0	0	0	0	0	0	0	0	17	8	0	0	0.33
	320	73	20	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	27	0	0	0	0.27
	640	76	12	0	0	0	5	0	0	0	0	0	0	6	0	0	0	0	18	0	0	6	0.42
4	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	80 ^a	82	4	0	4	0	9	0	0	0	0	0	0	0	0	0	0	0	13	0	4	0	0.25
	160	76	8	4	0	0	8	0	0	0	0	0	0	0	4	0	0	0	20	4	0	0	0.28
	320	70	10	10	0	0	10	0	0	0	0	0	0	0	4	0	0	0	20	4	0	0	0.40
	640	82	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0.18
40	0	35	0	0	0	0	45	10	0	0	0	0	0	5	0	5	0	0	45	15	5	0	0.90
	80 ^a	13	0	0	0	0	27	33	13	7	0	0	0	7	0	0	0	0	27	33	13	13	1.51
	160	4	4	0	0	0	30	22	11	4	0	0	4	11	4	0	7	0	37	22	22	15	2.07
	320	9	0	0	0	0	10	29	19	14	0	0	0	14	0	0	0	5	10	29	19	33	2.57
	640 ^a	0	0	0	0	0	7	13	27	20	0	0	13	7	7	0	0	7	13	13	40	33	2.91
Experiment II (7-1/2 Months Post Irradiation)																							
200	0	7	0	0	0	0	37	41	11	0	0	0	4	0	0	0	0	0	37	41	15	0	1.63
	80 ^a	8	0	0	0	0	25	50	4	0	0	0	8	0	0	0	4	0	25	50	16	0	1.75
	160	0	0	0	0	0	44	36	4	0	0	8	4	0	0	0	4	0	44	44	12	0	1.68
	320 ^a	0	0	0	0	0	38	30	4	4	0	0	12	0	0	4	4	0	38	34	20	4	1.82
	640	0	0	0	0	0	46	19	8	0	4	8	0	0	0	15	0	0	50	42	8	0	1.58

^aDue to rounding off of digits, sum of percent of rats does not equal 100

FIGURE 14

INCIDENCE OF RATS HAVING VARIOUS GRADED PROPORTIONS OF THE THYROID GLAND MASS OCCUPIED BY TUMOR TISSUE
EXPERIMENT I

^a Concentration of methimazole in the drinking water in parts per million (ppm)

FIGURE 15

INCIDENCE OF RATS WITH MEDULLARY THYROID TUMOR ONLY
HAVING VARIOUS GRADED PROPORTIONS OF THE THYROID MASS OCCUPIED
BY TUMOR TISSUE - EXPERIMENT I

^a Concentration of methimazole in the drinking water in parts per million (ppm)

FIGURE 16

way in Figure 17 which also includes the relative incidence of non-tumorous thyroids in each of the 40 ppm MMI groups. Figure 14 also shows that those thyroids of the 0 and 4 ppm MMI groups which contained tumors had a smaller degree of tumor involvement than did the tumorous thyroids of the 40 ppm MMI groups. This is shown in another way in Figure 18, where the mean of the graded thyroid tumor involvement for all the animals of an experimental group (including those with a grade of 0) is plotted against thyroid radiation dose. Figure 15 reveals that nearly all the thyroids with only medullary tumors regardless of the treatment group had a small proportion of the gland replaced by tumor (grade +1). Figure 16 shows that the few thyroids in the 0 and 4 ppm MMI groups which had non-medullary tumors only, had a small proportion of the gland mass occupied by tumor, in marked contrast to the situation in the 40 ppm MMI groups, where many of the thyroids exhibited a large proportion of tumor. Table 14 shows that malignant non-medullary tumors were associated in every case with a large proportion of tumor in the thyroid mass (grade +4). Table 14 also shows that thyroids containing both medullary and non-medullary tumors appeared: 1) infrequently; 2) primarily in the 40 ppm animals; and 3) without correlation with graded proportion of thyroid mass occupied by tumor.

FIGURE 17

INCIDENCE OF RATS HAVING VARIOUS GRADED PROPORTIONS
OF THE THYROID MASS OCCUPIED BY TUMOR TISSUE - EXPERIMENT I
(40 ppm MUI EXPERIMENTAL GROUPS)

FIGURE 18

MEAN GRADE OF THE PROPORTION OF THYROID MASS OCCUPIED BY TUMOR

B. Experiment II

All the rats in this experiment received MMI in the drinking water at a level of 200 ppm. In addition, each rat received localized thyroid irradiation of 0, 80, 160, 320, or 640 rads.

1. Survival data

Survival data for Experiment II are shown in Table 2. No significant trends in survival at 7-1/2 months post-irradiation are evident.

2. Change in Body Weight

Changes in body weight at 7-1/2 months post-irradiation, based on the data from sacrificed animals, are shown in Table 4 and Figure 2. All the treatment groups showed a loss of body weight. The size of the loss of body weight tended to decrease with increasing thyroid radiation dose, ranging from 20.9 grams for the 0 rad group, to 1.1 grams for the 640 rad group. One way analysis of variance suggests that the relationship between increasing thyroid radiation dose and decreasing body weight loss is significant ($p < .05$).

While comparing weights or weight changes between groups in Experiment I and groups in Experiment II must be done cautiously since in the two experiments the rats were of different ages at sacrifice, it is interesting to note that all the groups in Experiment II had weight losses, while all fifteen of the groups in Experiment I had weight gains.

3. Thyroid Assembly Weight

Thyroid assembly weights (thyroid and attached cut sections of trachea and esophagus) for the 5 groups of rats in Experiment II are shown in Table 5 and Figure 3. One-way ANOVA indicates that radiation had a very significant effect on thyroid assembly weight at the 200 ppm level of MMI treatment ($p < .001$). At the lowest radiation dose (80 rads) the assembly weight was slightly increased, while at the highest level of radiation (640 rads) the assembly weight was significantly decreased. The thyroid assembly weight/body weight ratios for Experiment II are shown in Table 6 and Figure 4. One-way ANOVA indicates that irradiation had a very significant effect ($p < .001$) on the ratio, increasing it at the lowest radiation dose and decreasing it at higher radiation doses.

4. Histology of Non-Tumorous Thyroid Tissue

The thyroids of the 200 ppm MMI rats resembled those of the 40 ppm MMI rats in the relative lack of colloid and in increased epithelial cell height. Foci of inflammatory cells were seen more frequently in the thyroids of the 200 ppm MMI treated animals than in the thyroids of the Experiment I animals. At this level of MMI treatment, no significant variation in thyroid gland appearance with radiation dose was observed.

A polyarteritis like lesion was seen more frequently in the 200 ppm MMI treated animals than in the 0, 4, or 40 ppm MMI treated animals. The incidences of rats with this lesion in their thyroids were 8/28 (39%), 5/22 (23%), 3/25 (12%), 2/25 (8%) and 0/25 (0%)

in the 0, 80, 160, 320, and 640 rads + 200 ppm MMI experimental groups, respectively, suggesting that the incidence of the lesion is decreased by irradiation. As in the rats of Experiment I, no evidence of the lesion was seen outside the thyroid area in any of the tissues examined.

Quantitative histologic results from Experiment II are shown in Tables 7-10 and Figures 5-8. The trend of increasing relative amounts of follicular epithelium and decreasing relative amounts of colloid content with increasing MMI dosage, which was seen in Experiment I, was continued with the still higher level of MMI dosage used in Experiment II, although Experiment II ran for 7-1/2 months, as opposed to the 13 months of Experiment I.

ANOVA showed a significant positive correlation between radiation dose and relative amount of connective tissue ($.01 < p < .025$). ANOVA did not show interaction between radiation dose and the other three quantified variables of interest: colloid, epithelial tissue, and vasculature ($.3, .9, .2, < p$ respectively), although the colloid component of the thyroid tissue monotonically decreased with increasing radiation dose (Table 8, Figure 6).

5. Thyroid Tumors

The data on rats with thyroid tumors in Experiment II was tabulated using the same classification scheme as in Experiment I, and is given in Table 15. The same types of thyroid tumors were seen in Experiment I. The incidences of benign, malignant, and medullary tumors in the 200 ppm MMI groups were comparable to the

incidences of these tumors in the 40 ppm MMI groups, except for the 0 radiation groups, where the incidence of rats with thyroid tumors in the 40 ppm MMI groups was 55%, while in the 200 ppm MMI group the incidence was 93% (Table 15). The benign thyroid tumors in Experiment II were in general more homogeneous in size than were the benign tumors in Experiment I. This may have been due to the shorter duration of Experiment II as compared to Experiment I (7-1/2 vs. 13 months). Another hypothesis which would explain the increased homogeneity of tumor size involves the relative strengths of the two treatments; MMI and radiation. Since all the Experiment II groups received MMI at the 200 ppm level, homogeneity of tumor size would be expected if this level of MMI was the major factor in creating benign thyroid tumors and promoting their growth; that is, if the benign thyroid tumorigenic effects of 80 to 640 rads of localized x-rays were small compared to the effects of 200 ppm MMI treatment. The incidence of rats with thyroids containing medullary and non-medullary tumors, and the incidence of rats with thyroids containing only non-medullary malignant tumors showed an increase in incidence with increasing radiation dose (Table 15). These trends, however, were not found to be significant on the basis of ANOVA ($.05 < p$). The proportion of the thyroid mass occupied by tumor in experiment II had no significant relationship to radiation dose (ANOVA $.05 < p$) (Table 14, Figure 18, Figure 19).

The excess incidence of rats in Experiment II with tumorous thyroids using the 200 ppm MMI + 0 rad group as the control is shown

TABLE 15

INCIDENCE OF RATS WITH THYROID TUMORS

EXPERIMENT II (7-1/2 MONTHS POST IRRADIATION,
200 ppm MMI)

Thyroid Tumor Type

Thyroid Radiation Dose (rads)	<u>Any Type</u>	<u>Medullary Only</u>	<u>Medullary + Non-Medullary</u>	<u>Non Medullary Benign</u>	<u>Non Medullary Malignant</u>
0	93 (25/27)	0	0 (0/27)	89 (24/27)	4 (1/27)
80	92 (22/24)	0	4 (1/24)	79 (19/24)	8 (2/24)
160	100 (25/25)	0	4 (1/25)	84 (21/25)	12 (3/25)
320	100 (24/24)	0	8 (2/24)	79 (19/24)	12 (3/24)
640	100 (26/26)	0	15 (4/26)	73 (19/26)	12 (3/26)
Grand Mean	97 (122/126)	0	6 (8/126)	81 (102/126)	10 (12/126)

FIGURE 19
INCIDENCE OF RATS WITH THYROID TUMORS HAVING VARIOUS
GRADED PROPORTIONS OF THE THYROID MASS OCCUPIED BY TUMOR TISSUE - EXPERIMENT II
200 ppm MMI

in Table 16. A no treatment control (0 ppm MMI + 0 rads) was not run with Experiment II. However, the low incidence of rats with thyroid tumors found in this treatment group in Experiment I at 13 months after onset of treatment (6%; 1 medullary tumor out of 17 thyroids) makes it unlikely that many rats with tumorous thyroids would have been found in a no treatment control group in Experiment II at 7-1/2 months post onset of treatment.

From Table 16 it can be seen that all the irradiated groups had a higher incidence of rats with malignant thyroid tumors than did the non-irradiated group. This difference in incidence, however, between the non-irradiated group and any of the irradiated groups was not statistically significant (one-way ANOVA $.05 < p$). It is interesting to note that the excess incidence of rats with thyroids containing both medullary and non-medullary tumors tended to increase with increasing radiation dose (4% at 80 rads to 15% at 640 rads). However, the increase is not significant as tested by one-way ANOVA ($p > .05$).

C. Serum Thyroxine Levels

The serum thyroxine levels in the rat groups of Experiment III (short-term 0-3 months after onset of treatment) are given in Tables 17 and 18 and are shown in Figures 20-27. The serum thyroxine levels in Experiment I (13 months post-irradiation) are given in Tables 19-21 and are shown in Figures 28-41.

The serum thyroxine levels in the non-treated control rats ranged from 3.5 to 5.0 $\mu\text{g}/100\text{ ml}$ during Experiment III and from

TABLE 16

PERCENT EXCESS INCIDENCE OF RATS WITH THYROID TUMORS

EXPERIMENT II (7-1/2 MONTHS POST IRRADIATION)

THYROID TUMOR TYPE

Thyroid Radiation Dose (rads)	<u>Any Type</u>	<u>Medullary Only</u>	<u>Medullary + Non-Medullary</u>	<u>Non Medullary Benign</u>	<u>Non Medullary Malignant</u>
0	-	-	-	-	-
80	-1	0	4	-10	4
160	7	0	4	- 5	8
320	7	0	8	-10	8
640	7	0	15	-16	8
Mean for all dose levels	5	0	8	-10	7

.6 to 6.0 $\mu\text{g}/100\text{ ml}$ during Experiment I. The fact that the 6.0 $\mu\text{g}/100\text{ ml}$ value is more than twice as large as the values in the month immediately preceeding and following it, and that it is almost 50% higher than the second highest value, makes that value suspect. If the 6.0 $\mu\text{g}/100\text{ ml}$ value is assumed to be in error, the range of serum thyroxine levels for the non-treated controls during Experiment I contracts to 2.6 to 4.2 $\mu\text{g}/100\text{ ml}$. No significant decrease in serum thyroxine level occurred with the passage of time in the non-treated control group. The average serum thyroxine value for the Experiment III non-treated control groups was 4.1 $\mu\text{g}/100\text{ ml}$. For the Experiment I non-treated control group, the average serum thyroxine value was approximately 3.7 $\mu\text{g}/100\text{ ml}$. The serum thyroxine levels in the 640 rads + 0 ppm MMI group in Experiment III were lower than the control values in 10 out of the 11 measurements made during the 3 months immediately post-irradiation. According to the Wilcoxon sum test for differences this difference in ranking is significant ($p < .01$).

As seen in Figure 31, the same 640 rad + 0 ppm MMI treatment group in Experiment I appears to have a lower serum T_4 level than the non-treated control groups until 10 or 12 months post-irradiation.

The other 0 ppm MMI long-term groups in Experiment I appear to have approximately the same serum T_4 levels as the non-treated control group. All of the short-term (Experiment III) 4 ppm MMI experimental groups seem to have nearly the same serum thyroxine levels as the non-treated control group, although there is a

suggestion that the 0 rad + 4 ppm MMI group has a slightly lower T_4 level beginning at three weeks after treatment (Figure 21). Data from the long-term study (Experiment I) show that at 8 of the 11 time points the serum T_4 level of the 0 rads + 4 ppm MMI experimental group was lower than the serum T_4 level of the non-treated control group (Figure 32). Likewise, in the long-term 80 rad + 4 ppm MMI and in the 160 rad + 4 ppm MMI groups (Figures 33 and 34) it appears that the serum T_4 levels of the MMI treated groups are lower than the serum T_4 levels of the non-treated control group. This is not the case, however, in the long-term 320 rads + 4 ppm MMI and 640 rads + 4 ppm MMI groups, where serum T_4 levels appear to be equal to those of the non-treated control group (Figure 35 and 36).

In general, the serum T_4 levels in the short-term (Experiment III) 40 ppm MMI groups dropped rapidly (starting by 1 week after the onset of treatment) below the control serum T_4 levels and remained well below the control values. Three exceptions to this general trend exist: 1) The 640 rads + 40 ppm MMI and the 320 rad + 40 ppm MMI groups have what appear to be spuriously high T_4 levels at 2 weeks post-irradiation. The fact that the groups at the two highest radiation doses have unusually high T_4 levels at the same point in time suggests the possibility of a delayed radiation effect. However, it is possible that the levels are due to errors in performing the assay; 2) The T_4 level for the 320 rads + 40 ppm MMI group increased from the 6th to the 12th weeks when it became equal to the control value; 3) The T_4 levels for the 160 rad + 40 ppm MMI group were approximately equal to those for the control group.

All the 40 ppm MMI long-term (Experiment I) groups show roughly the same pattern in their serum T_4 levels (Figures 37-41). At three months post-irradiation the T_4 levels were below the values for the non-treated control group. At four to six months post-irradiation the T_4 levels peaked at values close to those of the non-treated control group. Between six and seven months post-irradiation the levels fell to a plateau significantly below the control values, and remained at the new level for the duration of the experiment. The level of the plateau seemed to decrease slightly with increasing thyroid radiation dose (Table 20).

TABLE 17

MEAN^a SERUM T₄ RIA LEVELSShort Term, 0 and 4 ppm MMI Groups
μg/100 ml ± S.D.

Radiation dose + MMI level	Time Post Irradiation (weeks)											
	0	1	2	3	4	5	6	7	8	9	10	12
0 rads + 0 ppm	3.7 ± 0.3	4.2 ± 0.5	3.5 ± 0.6	3.5 ± 1.0	3.7 ± 1.1	4.3 ± 0.6	5.0 ± 1.6	4.3 ± 0.7	4.0 ± 0.8	4.1 ± 1.3	4.7 ± 1.0	4.2 ± 0.8
640 rads + 0 ppm	4.2 ± 0.6	2.2 ± 0.6	3.0 ± 0.6	2.6 ± 0.5	3.3 ± 0.6	3.2 ± 0.4	4.3 ± 0.8	3.2 ± 0.5	4.7 ± 1.0	3.5 ± 0.5	2.9 ± 0.5	3.4 ± 0.8
0 rads + 4 ppm	4.2 ± 0.3	3.6 ± 0.8	4.0 ± 1.2	2.6 ± 0.9	3.2 ± 0.8	4.0 ± 1.3	3.3 ± 0.9	3.8 ± 0.3	4.4 ± 0.9	4.9 ± 0.8	3.4 ± 1.2	3.3 ± 0.5
640 rads + 4 ppm	4.0 ± 1.1	4.7 ± 0.6	3.7 ± 0.9	4.1 ± 0.4	4.1 ± 0.6	4.4 ^b	4.9 ± 1.5	5.2 ± 1.3	3.9 ± 0.5	3.6 ± 0.4	3.6 ± 0.8	3.4 ± 0.9

^a4 rats per time point, unless otherwise noted^b1 animal only

TABLE 18

MEAN^a SERUM T₄ RIA LEVELSShort Term, 40 ppm MMI Groups
μg/100 ml ± S.D.

Treatment (Thyroid radiation dose + MMI level)	Time Post Irradiation (weeks)											
	0	1	2	3	4	5	6	7	8	9	10	12
640 rads + 40 ppm	3.6 ± 0.9	3.6 ± 1.7	5.2 ± 1.2	1.9 ± 0.6	2.7 ± 0.1	1.8 ± 0.2	2.4 ± 0.5	1.8 ± 0.3	2.0 ± 0.5	3.3 ± 0.4	1.9 ± 0.8	1.1 ± 0.2
320 rads + 40 ppm	4.0 ± 0.5	2.2 ± 0.2	3.6 ± 0.7	1.7 ± 0.4	2.4 ± 0.7	2.6 ± 1.3	1.8 ± 0.7	2.4 ± 0.8	3.1 ± 0.9	3.4 ± 1.6	3.6 ± 0.8	4.4 ± 0.7
160 rads + 40 ppm	3.9 ± 0.7	3.7 ± 0.8	3.5 ± 0.8	2.7 ± 0.7	3.6 ± 0.5	4.2 ± 0.6	3.4 ± 0.9	2.4 ± 0.4	4.2 ± 1.7	3.9 ± 0.4	4.2 ± 0.2	3.4 ± 0.5
80 rads + 40 ppm	4.0 ± 0.5	4.0 ± 0.3	2.8 ± 0.8	2.0 ± 0.2	2.4 ± 0.6	2.2 ± 0.6	2.3 ± 0.5	2.8 ± 0.7	2.9 ± 0.5	3.1 ± 0.9	1.8 ± 0.4	1.3 ± 0.4
0 rads + 40 ppm	4.1 ± 1.3	2.7 ± 0.3	2.6 ± 0.8	2.5 ± 0.7	2.5 ± 0.2	2.6 ± 0.2	2.4 ± 1.0	2.0 ± 0.3	2.1 ± 1.0	1.9 ± 1.0	1.9 ± 0.7	2.1 ± 0.5

^a 4 rats per time point

TABLE 19
MEAN^a SERUM T₄ RIA LEVELS
LONG TERM
0 ppm MMI GROUPS
μg/100 ml ± S.D.

Radiation Dose (rads)	Time Post Irradiation (months)										
	3	4	5	6	7	8	9	10	11	12	13
0	2.7±0.7	6.0±1.4	2.6±0.3	3.9±0.3	3.9±0.7	3.9±1.0	3.3±0.3	3.3±0.6	4.2±0.2	3.7 ^b	3.7±0.5
80	4.4±0.3	4.4±0.1	2.9±0.2	3.3±0.4	5.7±1.0	4.4±0.9	2.9±1.0	3.9±0.6	4.0±0.6	3.5±0.4	3.9±0.7
160	3.9±0.5	4.3±0.8	3.8±0.5	3.6±0.4	3.4±1.5	3.7±1.1	2.2±0.3	3.1±0.6	4.2±0.5	3.5±0.4	4.7±0.2
320	3.7±0.6	4.2±0.5	3.4±0.1	4.2±0.1	3.7±0.6	3.8±0.8	2.6±1.3	4.2±1.0	4.4±0.8	2.7±0.8	4.0±0.1
640	3.1±1.5	4.0±1.6	2.4±0.4	3.2±1.1	2.8±1.4	2.8±0.6	2.2±0.8	3.3±0.3	3.4±1.0	3.8 ^b	4.6±0.3

^a3 rats per time point, unless otherwise noted
^b1 rat

TABLE 20
 MEAN^a SERUM T₄ RIA LEVELS
 LONG TERM
 4 ppm MMI Groups
 µg/100 ml±S.D.

Radiation Dose (rads)	Time Post Irradiation (months)										
	3	4	5	6	7	8	9	10	11	12	13
0	2.4±0.3	1.7±0.7	3.6±0.2	4.2±0.1	3.2±1.3	3.4±0.8	1.6±0.7	3.8±1.7	3.7±0.8	2.7±0.6	3.1±1.6
80	2.9±1.0	3.5±0.2	3.6±1.0	3.1±1.8	2.6±1.6	3.8±0.6	2.5±0.6	3.4±1.0	1.6±0.4	2.85±0.6	2.8 ^b
160	1.9±0.3	3.6±0.8	3.5±0.4	3.4±0.3	2.9±1.3	3.0±1.2	2.7±0.8	2.4±0.5	3.3±0.4	3.2±0.6	4.8±2.9
320	2.9±0.8	5.9±1.5	4.2±0.8	3.0±0.6	3.3±1.5	3.0±1.0	3.6±0.4	3.9±1.5	2.8±0.7	3.4±0.2	3.2 ^b
640	2.4±0.6	3.7±1.1	3.2±0.4	4.0±0.6	2.5±0.1	3.7±0.8	3.6±1.5	4.5±0.6	3.75±0.5	2.8±1.1	4.1±1.1

^a3 rats per time point unless otherwise noted
^b1 rat

TABLE 21
MEAN^a SERUM T₄ RIA LEVELS
LONG TERM
40 ppm MMI Groups
μg/100 ml ± S.D.

Radiation Dose (rads)	Time Post Irradiation (months)										
	3	4	5	6	7	8	9	10	11	12	13
0	1.0±0.1	2.6±2.4	2.6±0.4	3.4±0.4	2.2±0.6	2.6±0.6	1.8±0.7	2.5±0.2	2.4±1.0	2.2±1.1	1.3±0.2
80	1.5±0.3	2.45±1.1	2.6±0.4	4.0±1.2	2.2±0.7	2.4±1.0	1.4±0.1	2.2±0.2	2.1±0.5	1.5±0.6	2.2±0.3
160	1.7±0.7	3.5±0.6	3.6±0.6	3.0±0.4	2.2±1.3	2.4±0.3	1.6±1.0	2.2±0.1	3.2±1.3	1.3±0.4	1.6±0.6
320	1.9±1.1	3.25±2.0	2.9±1.1	4.6±1.3	1.8±0.6	2.4±0.2	1.4±0.3	1.6±0.1	2.2±0.6	1.8±0.7	1.5±0.4
640	1.0±0.1	2.7±0.4	3.6±0.2	3.1±0.5	1.1±0.2	2.0±0.5	1.5±0.4	1.3±0.2	2.5±0.4	1.2±0.2	1.63±0.95

^a3 rats per time point, unless otherwise noted

FIGURE 20

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (640 rads + 0 ppm MMI)

FIGURE 21

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (0 rads + 4 ppm MMI)

FIGURE 22

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (640 rads + 4 ppm MMI)

FIGURE 23

MEAN RAT SERUM T_4 vs. TIME
SHORT TERM GROUP (0 rads + 40 ppm MMI)

FIGURE 24

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (80 rads + 40 ppm MMI)

FIGURE 25

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (160 rads + 40 ppm MMI)

FIGURE 26

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (320 rads + 40 ppm MMI)

FIGURE 27

MEAN RAT SERUM T_4 vs. TIME

SHORT TERM GROUP (640 rads + 40 ppm MMI)

FIGURE 28

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (80 rads + 0 ppm MMI)

FIGURE 29

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (160 rads + 0 ppm MMI)

FIGURE 30

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (320 rads + 0 ppm MMI)

FIGURE 31

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (640 rads + 0 ppm MMI)

FIGURE 32

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (0 rads + 4 ppm MMI)

FIGURE 33

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (80 rads + 4 ppm MMI)

FIGURE 34

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (160 rads + 4 ppm MMI)

FIGURE 35

MEAN RAT SERUM T_4 vs. TIME
LONG TERM GROUP (320 rads + 4 ppm MMI)

FIGURE 36

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (640 rads + 4 ppm MMI)

FIGURE 37

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (0 rads + 40 ppm MMI)

FIGURE 38

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (80 rads + 40 ppm MMI)

FIGURE 39

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (160 rads + 40 ppm MMI)

FIGURE 40
MEAN RAT SERUM T_4 vs. TIME
LONG TERM GROUP (320 rads + 40 ppm MMI)

FIGURE 41

MEAN RAT SERUM T_4 vs. TIME

LONG TERM GROUP (640 rads + 40 ppm MMI)

V. DISCUSSION

A. Thyroid Tumor Incidence

1. MMI Effect

The incidence of rats with thyroid tumors in all the 4 ppm MMI groups combined was approximately the same as in all the 0 ppm MMI groups combined (17 vs. 21%), suggesting that at this level of treatment MMI had no effect on total thyroid tumor incidence (Table 11). There was a slight difference in incidences of the types of tumors present in rats at these two treatment levels, in that 14% of the 0 ppm MMI rats had only medullary thyroid tumors while only 8% of the 4 ppm MMI rats had only medullary thyroid tumors (1% of the 4 ppm MMI rats had both medullary and non-medullary tumors in the thyroid). Although the difference in medullary tumor incidences (14% vs. 8%) is not significantly different (χ^2 , $0.1 < p$), the lower incidence of the MMI treated animals tends to support the conclusion of Doniach (1970) that medullary tumors are reduced by goitrogen administration.

The incidence of rats with thyroid tumors in the 40 and 200 ppm MMI groups was significantly greater than in the 0 and 4 ppm MMI groups (χ^2 , $p < .005$) (Tables 11, 15). The increase in total incidence was due primarily to the increased incidence of non-medullary tumors, although the 40 ppm MMI groups also had an increase in carcinoma incidence. In addition, the 40 and 200 ppm MMI groups had larger fractions of rats with both medullary and non-medullary thyroid tumors than did the 0 and 4 ppm MMI groups (χ^2 , $p .05$ for 40 ppm, $p < .10$ for 200 ppm).

The tumorigenic mechanism whereby the 40 and 200 ppm MMI treatments caused an increased incidence of thyroid tumors probably involves hormone imbalance. That is, the decreased levels of circulating thyroid hormone, caused by the MMI induced blockage of hormone synthesis, results in an increased level of TSH, which acts as a stimulus on the thyroid gland, causing increased metabolism, and eventually, gland hyperplasia. For reasons not completely understood, continued endocrine gland stimulation is capable of causing neoplasia (Furth 1975). However, the possibility that MMI or one of its metabolites is a carcinogen cannot be ruled out on the basis of these experiments.

The only significant incidences of non-medullary carcinomas occurred in the 40 ppm MMI groups, where the incidence of rats with thyroid carcinomas increased from 5% (1/20) at 0 rads to 20% (3/15) at 640 rads with intermediate incidence values at intermediate radiation doses (Table 11, Figure 13).

2. Radiation Effect

The effect of radiation on thyroid tumor production in the 0 to 4 ppm MMI groups was not significant. The non-irradiated 0 and 4 ppm groups did have a lower incidence of rats with tumors than the irradiated groups did, but within the irradiated groups, the size of the radiation dose seems to have made little difference (4 ppm MMI groups) or no difference at all (0 ppm MMI groups) in the incidence rate. A lack of an increase in the incidence of rats with thyroid tumors with increasing radiation dose is contrary

to what would be expected on the basis of current radiation biological theory, (Casarett 1965, 1973a, 1973b), which states that at low doses of radiation and low incidences of tumors, increasing radiation doses should increase tumor incidences. This theory is based on the assumption that the tumorigenic mechanism of ionizing radiation involves damage to the DNA of a cell, and that the number of damaged cells and the amount of damage per cell increases with the amount of radiation given to the cell, provided that the radiation dose is low enough to avoid excessive cell killing. Too high a radiation dose will result in cell loss due to radiation sterilization or cell killing, thus removing transformed cells from the surviving fraction and reducing the size of the pool of potential tumorigenic cells.

It is possible that the number of latent tumor cells increased with increasing radiation, but that conditions proper for the unmasking of the latent cells were not present. This speculation follows from the two stage hypothesis of tumorigenesis, implying that the initiating conditions were met by radiation exposure, while the promoting conditions were not completely fulfilled. This line of thought is supported by the tumor incidences observed in the 40 ppm MMI groups. At this level of MMI, the lowest radiation group (80 rads) had a higher incidence than did the no radiation group (87% to 65%). Also, the groups with higher levels of radiation (160, 320, 640 rads) had a larger combined incidence of rats with tumorous thyroids (60/63) than did the 0 and 80 rad groups (26/35) ($\chi^2 = 9.2$, $p < .01$).

The fact that the 160, 320 and 640 rad groups had all approximately the same incidence of rats with thyroid tumors need not imply that tumor incidence does not increase with increasing radiation dose. Rather, it may be a reflection of the fact that the tumor incidence was so high in the 160 rad group, that 'saturation' of tumor incidence was occurring, so that increasing the radiation dose could do little to increase the incidence of rats with tumors.

Consistent with the idea of increasing tumorigenic potential with increasing radiation dose in the dose range examined is the fact that the extent of the thyroid replaced by tumor increased with increasing radiation dose for the 40 ppm MMI groups (Table 14, Figures 14-18). This observation suggests that increasing the radiation dose did have an effect on tumor progression, causing either 1) an earlier appearance of tumors while at least maintaining the tumor growth rate, 2) an increased number of tumors per gland, 3) an increased growth rate of tumors, or 4) some combination of the above.

B. Quantitative Histology

1. MMI Effects

Most of the changes observed in the thyroid gland through the use of quantitative histology can be attributed to the action of MMI on the gland. The MMI induced blockage of the binding of iodine atoms to organic molecules in the thyroid epithelial cells results in a decreased synthesis of thyroid hormone by the gland. This decrease in synthesis of thyroid hormone, coupled with the

requirement of physiological amounts of the hormone for normal body function, causes the gland to draw upon the stored hormone in the colloid to meet the needs of the body. Therefore, the gland's store of hormone--the colloid--is reduced. A decrease in the relative amount of colloid present was clearly evident in the comparison of the quantitative histology data for the 0 ppm MMI experimental groups with the data for the 4, 40 and 200 ppm MMI groups. Although direct comparisons between the 7-1/2 month duration 200 ppm MMI experiment groups and the 13 months duration 0, 4 and 40 ppm MMI groups must be made with caution, it is interesting that at every level of radiation used, the relative amount of colloid present in the thyroid gland decreased with increasing MMI dose. This can be explained by assuming that the degree of blockage of thyroid hormone synthesis is a function of the level of MMI administered. That is, the 4 ppm MMI thyroids experienced a relatively small block and only had to draw upon a small portion of the stored hormone to maintain thyroid hormone levels, while the 40 and 200 ppm MMI thyroids experienced relatively larger hormone synthesis blocks, and had to draw upon a greater amount of their hormone stores.

The fact that the quantitative histology data was recorded as relative area of the thyroid gland occupied by various tissue components implies that the decrease in relative colloid area with increasing MMI dose must be balanced by an increase in the relative areas of one or more of the other tissue components. Such an increase in relative area with increasing MMI dose is in fact seen.

in the follicular epithelial component. Some of this increase may be due to the decrease in colloid; but some is probably due to the effects that increased levels of TSH (due to the decreased levels of T_4) have on the follicular epithelial cells. These effects, as outlined by Tong (1974) in his review on the actions of TSH on thyroid cells probably begin with the elevation of adenylyclase activity within minutes after an increase in TSH levels, continue through various alterations in intermediate thyroid metabolism and culminate in increased mitotic activity. Hypertrophy from increased numbers of mitochondria and other cellular components, combined with hyperplasia as a result of increased mitotic activity combine to increase the relative area of the thyroid occupied by follicular epithelial cells.

Quantitative histology determined that MMI treatment increased the relative amount of vasculature. This increase may have been in part or in total due to the relative decrease of colloid. However, it has previously been suggested that methimazole treatment increases the vascularity of hyperplastic human thyroid glands (DeCourcy, 1952) and that at least three goitrogens (methylthiouracil, carbimazole and perchlorate) are capable of increasing thyroid blood flow within days after the onset of treatment (Kapitola, et al., 1971). The increase in blood flow is suppressible through the administration of dessicated thyroid powder (Kapitola et al., 1974) suggesting that the blood flow effect is presumably related to elevated TSH levels. It is conceivable that increased levels of metabolic wastes arising

as a result of TSH mediated increased thyroid metabolism, act as the signal for increased blood flow, and that the same signal, if continued could act as a stimulus for increased vascularization of the thyroid gland.

2. Radiation Effects

Quantitative histology revealed that radiation significantly increased the relative area of connective tissue in the thyroid. This is in agreement with previous observations that increased fibroblastic activity follows exposure to significant levels of radiation (Rubin and Casarett, 1968). The initiating event for the fibroblastic activity is believed to be the radiation induced increased permeability of small blood vessels which leads to interstitial edema. It is interesting to note that at 13 months post-irradiation, the experimental group which received only 80 rads of radiation to the thyroid had a significantly greater relative amount of connective tissue than did the non-treatment control (t^2 , $p < .05$). Thus, unless the relative increase is due to an absolute decrease in one of the other tissue components, it appears that fibroblastic proliferation can occur at a level of radiation which does little significant damage to the surrounding parenchymal tissue. The evidence of increased connective tissue at low radiation levels agrees with the conclusion of Marks et al. (1957) that,

"Although the epithelial effects (of daily feeding of 5 μ Ci or more of 131 I to sheep) are probably responsible for the functional decline of the gland, the diagnosis of early thyroid damage due to prolonged exposure to 131 I at low levels would best be attempted

on the manifestations in the interstitial tissue... Interstitial fibrosis was the prominent feature of the pattern of damage in the adult animal. It may be assumed that the fibrosis represents the end stage of preceeding inflammatory or necrotizing processes. However, at the lowest injurious level, the group fed 5.0 μc of I^{131} daily, the earlier stages postulated were not detectable, so that the fibrosis in effect assumed the significance of an initial indicator of damage."

The variation in relative amount of colloid with increasing radiation dose is difficult to explain. It is possible that the relative amount of colloid decreases at the high radiation levels because the relative amount of follicular epithelial tissue increases. The relative amount of follicular epithelial tissue may be increased at the higher radiation levels due to radiation damage to the tissue. The radiation damage may impair iodine transport, hormone synthesis or hormone storage so that the epithelial cells undergo hypertrophy and/or hyperplasia as part of a compensatory reaction. This reaction may be mediated by TSH, or may arise as a result of the auto regulatory mechanism. In the 4 ppm MMI + 80, 160 and 320 rad groups, and in the 40 ppm + 80 and 160 rad groups the relative amount of colloid was greater than in the appropriate MMI no radiation control. No increase in colloid with low levels of radiation as compared to the zero radiation control was seen in the 200 ppm MMI groups. A possible pattern can be discerned in these results. When the thyroid is severely stressed (200 ppm MMI) low levels of radiation do not increase the relative amount of colloid. When the thyroid is moderately stressed (40 ppm MMI), low levels of radiation (80-160 rads) can slightly increase the relative amount

of colloid, as compared to the non-irradiated control. When the thyroid is slightly stressed (4 ppm MMI) low to moderate levels of radiation (80-320 rads) can increase the relative amount of colloid. At the 4 ppm MMI level, the peak increase in relative colloid occurs at a higher radiation dose, and is larger, both in terms of absolute and per cent increase over the non-irradiated control than in the 40 ppm MMI level. The same pattern of increased colloid (with respect to non-irradiated control) with low levels of radiation does not appear when the thyroid is not goitrogen stressed (0 ppm MMI). Two possible hypotheses for this pattern appear to exist. 1) The effect in some way requires the presence of MMI, a metabolite of MMI or some response to MMI. 2) The value for the relative amount of colloid in the 0 rad + 0 MMI group may be too high. If the estimate of the relative amount of colloid in the 0 rad + 0 MMI group was one standard deviation below the measured value, the same pattern of increasing colloid with low doses of radiation would be present. If we assume the second hypothesis is true, we are left with the question, "What causes the increase in relative colloid at low doses of radiation, an increase which diminishes with increasing MMI dose?" However, since the quantitative histological measurements were based on relative areas rather than absolute areas, and since the colloid is made up only of non-living matter while the other three tissue components studied are made up of living cells, it may be more reasonable to assume that the colloid relative area changed passively, as a consequence of the response to radiation

of the other tissue components. Indeed, the plot of relative follicular epithelial area (Figure 7) is almost a mirror image of the plot of relative colloid (Figure 6), showing decreases at low levels of radiation for the 4 and 40 ppm MMI groups. A decrease in relative follicular epithelial tissue is also seen for the 200 ppm MMI group at the 80 rad level. And, if the relative follicular epithelial area of the 0 rad + 0 MMI group were to be increased by one standard deviation (the mirror reflection of what was discussed for the 0 rad + 0 MMI colloid area), the same pattern of a decrease of follicular epithelium with low doses of radiation would also appear. Therefore, a possible explanation of the fluctuation in relative colloid area at low doses of radiation is that it is the result of changes in the relative follicular epithelium area.

Radiation tended to decrease the relative amount of vasculature in the MMI treated animals (Table 10). This effect was not seen in the 0 ppm MMI group. A possible explanation for this effect is found in the fact that MMI treatment tends to increase thyroid gland size and vasculature. If, due to a differential radiation sensitivity, the vasculature is unable to grow as rapidly as the other tissue components, the relative amount of vasculature in the thyroid would decrease.

The relative amount of vasculature decreased slightly at the high radiation levels. This decrease may reflect the increase in the relative amount of connective tissue, or it may be an absolute change. The decrease in relative amount of vasculature with increasing

radiation dose is more pronounced in animals receiving the 40 ppm MMI treatment than in the animals receiving the 0 to 4 ppm MMI treatment. This suggests that the decrease in relative amount of vasculature may be caused by an inability to respond to a MMI related stimulus.

C. Serum T₄ Levels

The mean serum T₄ levels in the non-treated control groups in the short term and long term experiments were 4.1 ± 0.5 and 3.7 ± 0.9 $\mu\text{g}/100$ ml. (If the serum T₄ levels of the first three time points of the long term non-treatment control group (2.7, 6.0, 2.6 $\mu\text{g}/100$ ml) are ignored, the long term mean remains the same, but the standard deviation (S.D.) is reduced to one third its previous value -- 3.7 ± 0.3 $\mu\text{g}/100$ ml. The fact that the three ignored data points are more than 3 S.D. away from the recalculated mean suggests that in this case the procedure of ignoring the data points is reasonable.) These serum T₄ levels are comparable to those measured by Esber et al. (1974) in six strains of laboratory rats using 2 month old female rats (range 1.9-4.4 $\mu\text{g}/100$ ml, mean = 3.0 $\mu\text{g}/100$ ml). It is interesting that in only two of the strains measured, were the serum T₄ values greater than 3.3 $\mu\text{g}/100$ ml. The two strains with the highest serum T₄ levels (3.9 and 4.4 $\mu\text{g}/100$ ml) were the only two

strains in the study which were derived from Wistar rats. These serum T_4 levels were reported to be significantly higher ($p < 0.01$) than the serum T_4 levels of the other strains tested, suggesting that at this age at least, Wistar derived female rats may have relatively higher serum T_4 levels than other comparably aged female rats.

Both Lu (1976) and Riesco et al. (1977) reported observing a decrease in serum T_4 levels in male rats when observed over approximately a one year period. Such a decrease was not seen in this experiment. However, the following differences between this experiment and the work of Lu and Riesco et al. may explain the variation in results: 1) This experiment used female rats, the other two used male rats. The variation in long term serum T_4 levels may be due to differences in secretion of sex related hormones. 2) Riesco et al. placed animals on an iodine deficient diet, supplemented with KI. It has been reported (Riesco et al. 1976) that commercially available iodine deficient diets are nutritionally deficient. Therefore, the drop in serum T_4 reported by Riesco et al. may be due to malnutrition. 3) Lu changed the method of serum T_4 assay during the course of his experiment, so that some of the decrease seen by Lu may be due to differences in assay selectivity and sensitivity.

The fact that of the 0 ppm MMI groups only the 640 rad group revealed lower than control values of serum T_4 suggests that the effect of radiation at the lower doses was insufficient to degrade

thyroid performance (Tables 17 and 19, Figures 20 and 31). Whether the apparent decrease in serum T_4 at 640 rads is real is not clear. No rigorous statistical tests can easily be applied to the long term data, due to the method of sample selection, which consisted of selecting at random 3 rats from those available for testing. Since animals died during the experiment, it was not possible to use the same animals for T_4 experiments at every time point, which would have satisfied one type of statistical test. Since the total number of animals used in each experimental group was moderate, it was not possible to use different animals for serum T_4 measurements at each time point, which would have satisfied another type of statistical test. For the short term serum T_4 values, for which the same animals were measured at each time point, application of the student t test at each time point to the 640 rad + 0 ppm MMI and 0 rad + 0 ppm MMI data shows that only 4 of the differences in mean serum T_4 levels (those at 1, 5, 7 and 10 weeks post-irradiation) were statistically significant. In all 4 of these cases, the irradiated group had the lower T_4 level. These test results may tend to support the hypothesis that 640 rads of thyroid radiation lowers serum T_4 levels, but they do not conclusively prove the hypothesis.

Data which can be interpreted as not supporting the hypothesis come from the serum T_4 measurements of the 640 rads + 4 ppm MMI group (Tables 17 and 20, Figures 22 and 36). In this experimental group, no evidence is seen of lowered serum T_4 levels, as compared to those of the non-treated control group. That MMI, a blocker of

thyroid function, would be able to elevate serum T_4 levels lowered by thyroid radiation seems paradoxical. It seems more probable that MMI at the 4 ppm level does not significantly alter serum T_4 levels (a hypothesis supported by the mean serum T_4 levels of all the other 4 ppm MMI groups), and that 640 rads of thyroid radiation also has no effect on serum T_4 levels.

All of the 40 ppm MMI experimental groups showed significant decreases in their mean serum T_4 levels as compared to either non-treated control or irradiated control groups (Tables 18 and 21, Figures 37-41). Apparently, at this level of treatment, MMI was able to significantly block thyroid hormone synthesis. During months 4-6 post irradiation, it appears that the serum T_4 levels of the 40 ppm MMI treated rats increased to near control levels and then decreased. The increase may reflect the attempt of the gland to overcome the MMI block via hyperplasia and hypertrophy. However, the time course of the action is puzzling. Christov (1975) reported that rat thyroid glands showed an increased weight and mitotic index within a week after beginning the administration of 0.1% MTU in the drinking water. The increased mitotic activity reached a peak at approximately 10 days after the beginning of treatment. Thereafter it decreased, although it was still elevated over pretreatment levels at 20 days after the beginning of treatment. From Christov's data it would seem surprising that the serum T_4 levels would remain depressed for three months before becoming elevated. The decrease from the elevated level is also puzzling, since it occurs in all the

40 ppm MMI groups at the same time, 6 to 7 months post-irradiation. It is unlikely that the decrease is due to the appearance of tumors, since 1) the first thyroid tumor observed in a rat dying spontaneously did not appear until 10 months post-irradiation (640 rads + 40 ppm MMI animal), 2) less than two-thirds of the 0 rad + 40 ppm MMI animals had thyroid tumors at 13 months post-irradiation, so that the probability of selecting only animals from that group which had non-functioning thyroid tumors at 7 months post-irradiation is small, 3) the tumors in the 0 rads + 40 ppm MMI group were relatively small at 13 months post-irradiation, so it is unlikely that any tumors present 7 months post-irradiation would be sufficiently large to alter serum T_4 levels.

It is conceivable that although an inter assay standard was run with every assay, the assays at months 4, 5, and 6 gave elevated serum T_4 readings. The standard used was at a fairly elevated level of T_4 (7.8 $\mu\text{g}/100\text{ ml}$) so that it is possible that deviations in the assay at low levels of T_4 would not be detected. The fact that the 0 and 4 ppm MMI groups do not show elevated readings in the same assays may mean that the assays did not give elevated readings, or it may mean that the assays gave elevated readings only on samples with serum T_4 levels lower than those found in the 0 and 4 ppm MMI groups.

D. Relative Risk, Doubling Dose and Absolute Risk

In its study of the somatic effects of ionizing radiation, the BEIR Committee (NAS-1972) defined the terms relative risk (R^2),

doubling dose (D^2) and absolute risk (AR). Relative risk is the ratio of the risk of an event occurring after irradiation compared to the risk of the event occurring without irradiation. As such, relative risk is calculated as the ratio of the incidence in the exposed population to the incidence in the control population. Doubling dose is defined as the amount of radiation needed to double the natural incidence of a genetic or somatic anomaly. Absolute risk is the number of cases of an anomaly that will result from the exposure of a given population. Absolute risk is calculated as the product of the assumed relative risk times the total population at risk.

Mathematically, the BEIR committee defined relative risk, doubling dose, and absolute risk as follows: when based on the experience of an irradiated group with that of a non-irradiated group, and using the linear hypothesis,

$$R^2 = \frac{\frac{X}{P}}{\frac{Y}{Q}}$$

$$D^2 = \frac{D}{R^2 - 1} \quad (\text{if } > 0)$$

$$AR = \frac{X - \frac{P \cdot Y}{Q}}{D \cdot P} \quad (\text{if } > 0)$$

where D = average radiation dose, X = number of anomalies in irradiated group, Y = number of anomalies in the non-irradiated group, P = the number of person-years in the irradiated group, and Q = the number of person-years in the un-irradiated group.

Modifying the definitions slightly so that x and y refer to tumors, and P and Q refer to rat months rather than person years, then the formulas can be applied to the data from the sacrificed animals in experiment I. Table 22 shows the calculation of R^2 , D^2 , and AR for each of the treatment groups, using both 0 rads + 0 ppm MMI and 0 rads + the groups' corresponding level of MMI as control. Note that since the thyroid tumor incidence of the 0 rad + 4 ppm MMI experimental group was zero, relative risk and doubling dose calculations cannot be performed using the group as a control population, since such calculations would require divisions by zero. Doubling dose and absolute risk estimates cannot be calculated for the 0 rad experimental groups, since such calculations would also entail divisions by zero. Relative risk as a function of radiation dose is shown in Figure 42. Absolute risk as a function of radiation dose is shown in Figure 43. At all three levels of MMI, and using either control (0 rads + 0 MMI and 0 rads + corresponding MMI), the absolute risk of thyroid tumor induction decreased with increasing radiation level. This suggests that the efficiency of tumor induction decreased with increasing radiation dose.

The low values of the doubling doses for the 40 ppm MMI groups when 0 rads + 0 MMI is used as control are results of the MMI effect at this level. The effect of the 40 ppm MMI treatment on the doubling dose can be seen by looking at the 40 ppm MMI doubling doses when calculated using the 0 rads + 40 ppm MMI group as control. With the effects of MMI removed, the doubling doses are more than

Table 22

Relative Risk, Doubling Dose, And Absolute Risk
For Rat Thyroid Tumor Incidence

Relative Risk (R^2)
Doubling Dose (D^2 , rads)
Absolute Risk (AR, 10^{-5} tumors/rad/rat month)

Control population taken as 0 rads + 0 ppm MMI group

Experimental Group Radiation (rads) MMI(ppm)		R^2	D^2	AR
0	0	1.0	-	-
80	0	3.0	40	14
160	0	3.7	60	10
320	0	4.8	80	7
640	0	4.2	200	3
0	4	0	-	-
80	4	2.3	60	10
160	4	3.0	80	8
320	4	5.1	80	8
640	4	3.1	300	2
0	40	9.4	-	-
80	40	14.7	6	100
160	40	16.4	10	57
320	40	15.4	22	26
640	40	17.0	40	15

Control population taken as 0 rads + 4 ppm MMI

80	4			17
160	4			11
320	4			9
640	4			3

Control population taken as 0 rads + 40 ppm MMI

80	40	1.6	140	69
160	40	1.7	210	34
320	40	1.6	490	11
640	40	1.8	780	7

FIGURE 42
RELATIVE RISK OF RAT THYROID TUMORIGENESIS

FIGURE 43
ABSOLUTE RISK OF RAT THYROID TUMORIGENESIS

10 times larger than when 0 rads + 0 MMI was used as control. The fact that the doubling doses for the 0 and 4 ppm MMI groups were approximately the same (40 to 80 rads) with the exception of the 640 rad groups (200 rads for 640 rads + 0 ppm group, 300 rads for the 640 rads + 4 ppm group) suggests that for low to moderate levels of radiation, the doubling dose was approximately constant, but that at higher doses the doubling dose increased. This conclusion tends to agree with the data from the AR calculations, which suggest that low radiation doses were more effective in tumor production on a per-rad basis, than high radiation doses. The relative risk of thyroid tumor induction for the 40 ppm MMI + radiation groups, did not increase significantly with increasing radiation dose, but remained fairly constant. This constancy probably arises from the fact that the 80 rads + 40 ppm MMI tumor incidence is so large (13 of 15 animals, or 87%), the addition of more radiation could not increase the incidence greatly.

VI. CONCLUSIONS

1. Radiation alone caused an increase in the incidence of rats with thyroid tumors. For the number of animals used in this study, the increase was not found to be statistically significant at the .05 level.
2. The low level of MMI used (4 ppm chronically administered in the drinking water) did not significantly alter the incidence of rats with thyroid tumors after 13 months of treatment, as compared to the non-treatment control group. This level of MMI treatment given to rats exposed to localized thyroid irradiation did not significantly increase the incidence of rats with tumorous thyroids, as compared to irradiated non MMI treated rats.
3. The incidence of rats with thyroid tumors after 13 months of treatment in the groups that received the moderate dose of MMI (40 ppm in the drinking water) was considerably greater than in the 0 and 4 ppm MMI groups. In each of the irradiated 40 ppm MMI treated groups, the incidence of rats with thyroid tumors showed an increase over the incidence in the 40 ppm MMI only group. The increase in incidence was found to be statistically significant by the χ^2 test at the .05 level for all radiation dose levels except the lowest.
4. The size of the thyroid tumor incidences in the treatment groups receiving both localized irradiation and 40 ppm MMI was greater than would be expected on the basis of simple addition of the incidences of each treatment given separately. That is, the treatments appeared to act in a positive synergistic manner.

5. Non-medullary thyroid carcinoma incidence was significantly greater in the 40 ppm MMI treatment groups than in the 0 or 4 ppm MMI treatment groups (χ^2 , $p < .05$). Within the 40 ppm MMI groups, non-medullary thyroid carcinoma incidence increased with increasing radiation dose, although the increase was not judged to be significant by the χ^2 test at the .05 level.
6. Localized thyroid irradiation in the range of 80 to 640 rads of female Rochester ex-Wistar rats had little effect on serum T_4 levels when observed to 13 months post irradiation.
7. Treatment with the low level (4 ppm in the drinking water) of the goitrogen methimazole by itself or in combination with 80 to 640 rads of localized thyroid irradiation produced no significant effect on serum thyroxine levels in female Rochester ex-Wistar rats out to 13 months after onset of methimazole treatment.
8. Treatment with the moderate (40 ppm in the drinking water) level of the goitrogen methimazole caused a decrease in the serum thyroxine levels of Rochester ex-Wistar female rats within 1 to 3 weeks after the onset of the treatment. The decrease was observed out to 13 months after onset of treatment. The decrease in serum thyroxine seemed to be positively correlated with thyroid radiation dose, being greatest for the 640 rad + 40 ppm group, and least for the 0 rad + 40 ppm group.
9. Quantitative histologic examination permitted detection of thyroid histologic alterations at the lowest levels of radiation exposure and methimazole treatment used in this study. Alterations

caused by radiation in the mean relative area of non tumorous thyroid tissue included 1) an increase in connective tissue, and 2) a slight decrease in vascular tissue. MMI treatment decreased the mean relative amount of colloid, while increasing the mean relative amount of vasculature and follicular epithelium.

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