Separate Summary Submitted with Thesis

For Degree of Ph.D. (Faculty of Medicine),

University of Glasgow, 1970.

TITLE OF THESIS

Radiation Effects on Thyroid Hormonogenesis and Cell Proliferation *in vivo*

CANDIDATE

William Rattray Greig, M.D. (Aberd.), M.R.C.P. (Edin.),

Senior Wellcome Clinical Research Fellow,

University Department of Medicine,

Royal Infirmary, Glasgow.

This thesis describes and compares some of the effects of external X-irradiation, iodine-131 and iodine-125 irradiations on the hormonogenetic functions of the thyroid and on cell proliferation in this tissue. All the studies were conducted *in vivo* and mostly on the rat whose thyroid is a suitable model for controlled experiments; observations were, however, also made on patients undergoing radiation treatment for thyrotoxicosis. The laboratory and clinical studies (conducted by myself) repeat, complement and supplement observations made by others. Appropriate reference is always made when investigations by others are discussed.

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radiations which led me to compare their effects on rat thyroid hormonogenesis and on cell proliferation respectively.

In Section C the studies of the effects on cell hormonogenesis are described. Cell hormonogenesis refers to thyroglobulin synthesis, thyroglobulin iodination, together with thyroglobulin composition, resorption, proteolysis and hormone release by follicular cells in vivo. The methods used measured tritiated leucine and radioiodine incorporation into ultracentrifugation fractions of thyroid proteins together with radiochromatographic analyses and measurement of rat blood hormone concentrations. The studies show that most aspects of thyroid hormonogenesis are relatively radio-resistant and this includes radio-resistance to iodine-125 irradiations.

In both sections D and E the effects of X-irradiation, iodine-131 or iodine-125 irradiations on follicular (and stromal) cell proliferation are described. The methods used included the promotion of cell proliferation in the rat thyroid by goitrogenic stimulation together with measurements of the proportion of cells in S-phase and of the D.N.A. synthetic process itself, using tritiated thymidine. The work, including special emphasis on iodine-125 effects, shows that interruption of cell proliferation, or impairment of cell survival, arises through the damage to the cell nucleus irrespective of damage to other parts of the cell.

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Dr William R. Greig - thesis no. 3413 - Ph.D.

To Betty, Steven, Susan and Louise Greig.

TITLE

Radiation Effects on Thyroid Hormonogenesis and Cell Proliferation *in vivo*.

SHORT TITLE

Radiation Effects on Thyroid

by

William Rattray Greig, M.D., (Aberd.), M.R.C.P. (Edin.)

Wellcome Clinical Research Fellow,

University Department of Medicine,

Royal Infirmary, Glasgow.

Submitted for Degree of Ph.D. (Faculty of Medicine),

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PREFACE AND SUMMARY

When Roentgen discovered x-rays it was appreciated that there would be a continuing demand for information about the biological and clinical effects of ionising irradiations. This demand is even more urgent now because of the great increase in the medical, industrial and military applications of a wide variety of ionising irradiations.

This thesis describes and compares some of the effects of external X-irradiation, iodine-131 and iodine-125 irradiations on the hormonogenetic functions of the thyroid and on cell proliferation in this tissue. All the studies were conducted *in vivo* and mostly on the rat whose thyroid is a suitable model for controlled experiments; observations were, however, also made on patients undergoing radiation treatment for thyrotoxicosis. The laboratory and clinical studies (conducted by myself) repeat, complement and supplement observations made by others and cited in medical and scientific literature. Appropriate reference is always made when investigations by others are discussed. I have not, however, included a comprehensive review of all the literature.

The thesis (composed by myself) consists of six sections, A, B, C, D, E and F. Section A is an introduction and contains a description of the microanatomy of the adult rat thyroid and the relation between minute structure and hormonogenesis and cell proliferation respectively. Section B contains a detailed account of the principles and procedures employed in the delivery and

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Dr. F.C. Gillespie and Mr. J.S. Orr, Principal Physicists in the Department of Clinical Physics and Bioengineering, Western Regional Hospital Board always gave me advice when it was sought and I am especially indebted to them for their help with some aspects of the dosimetry of iodine-131 and iodine-125.

I am also indebted to Dr. J.A. Thomson, Dr. I.T. Boyle, Dr. J.F.B. Smith, Dr. H.W. Gray, Dr. A. Lang, Mrs. Joyce Lang, Mrs. Jean Veitch and Mr. C. Foster who helped me with some of the laboratory and clinical studies. Mrs. Brenda Burn and Mr. Paul Kent prepared the photographic prints and Miss Irene Muir typed the thesis. I convey my thanks to them for their help.

CONTENTS

SECTION A

INTRODUCTION:

STRUCTURE AND FUNCTION OF RAT THYROID IN RELATION TO CURRENT RADIATION STUDIES

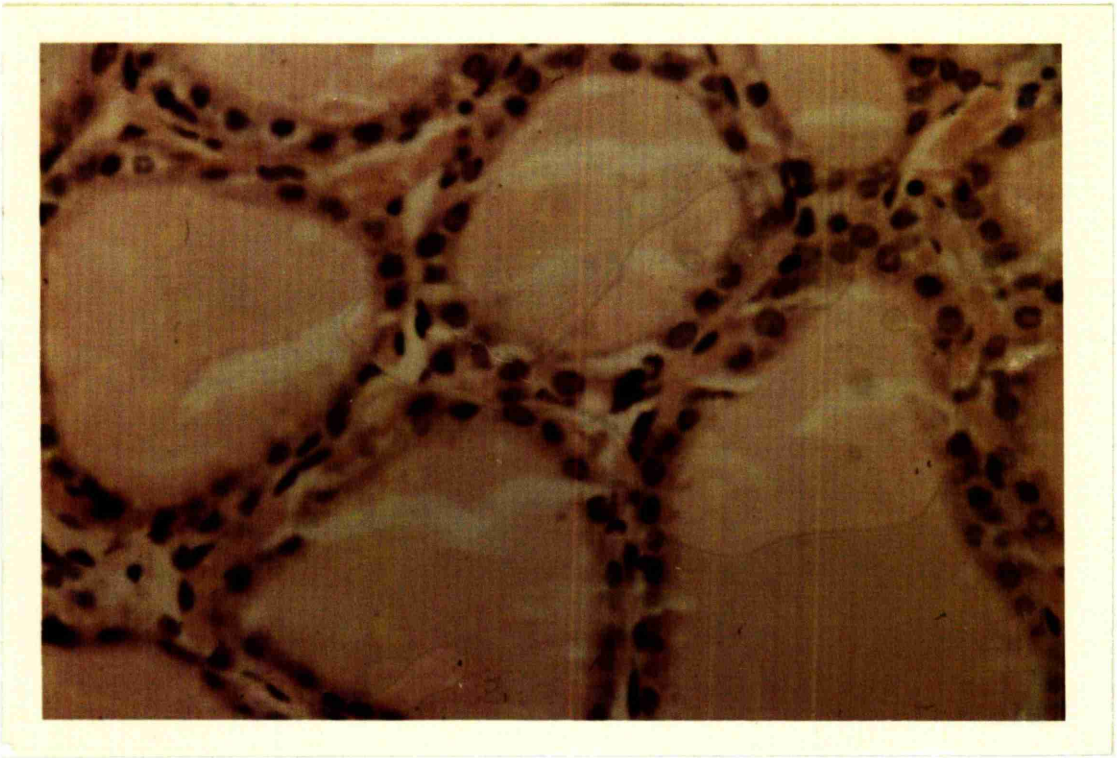


FIG. A-1 Normal rat thyroid (x400).

Knowledge of the microscopic and ultramicroscopic topography of the structures and the mechanisms controlling thyroid cell hormonogenetic functions and cell proliferation is relevant to defining the radiation dose of radioactive iodines and to understanding the nature of radiation effects, and how these are measured. In this thesis the effects of external X-irradiation, iodine-131 irradiations and iodine-125 irradiations on rat thyroid cell hormonogenesis and proliferation *in vivo* are compared.

STRUCTURE - GENERAL

Details of the structure of the rat thyroid have been studied and reviewed by Gross (1957), Wissig (1964), Ekholm (1964), Heimann (1966), Klinck (1964) and by Doniach (1967).

The thyroid gland of the normal adult rat has two lobes, tapering ellipsoids, joined by a slender isthmus, and the whole gland *in vivo* weighs about 15 - 25 mg. The parenchymal unit is the follicle, which is a spherical shell of follicular cells surrounding a lumen. The whole lumen is filled with colloid. (Fig.A-1). Groups of follicles form a lobule and groups of lobules form a lobe. The interfollicular and interlobular tissue is capillary arterioles and venules, lymphatics and a supporting stroma of collagen, elastic tissue and stromal cells. There are small groups of cells in the interfollicular volume. These highly specialised cells which secrete calcitonin are called parafollicular

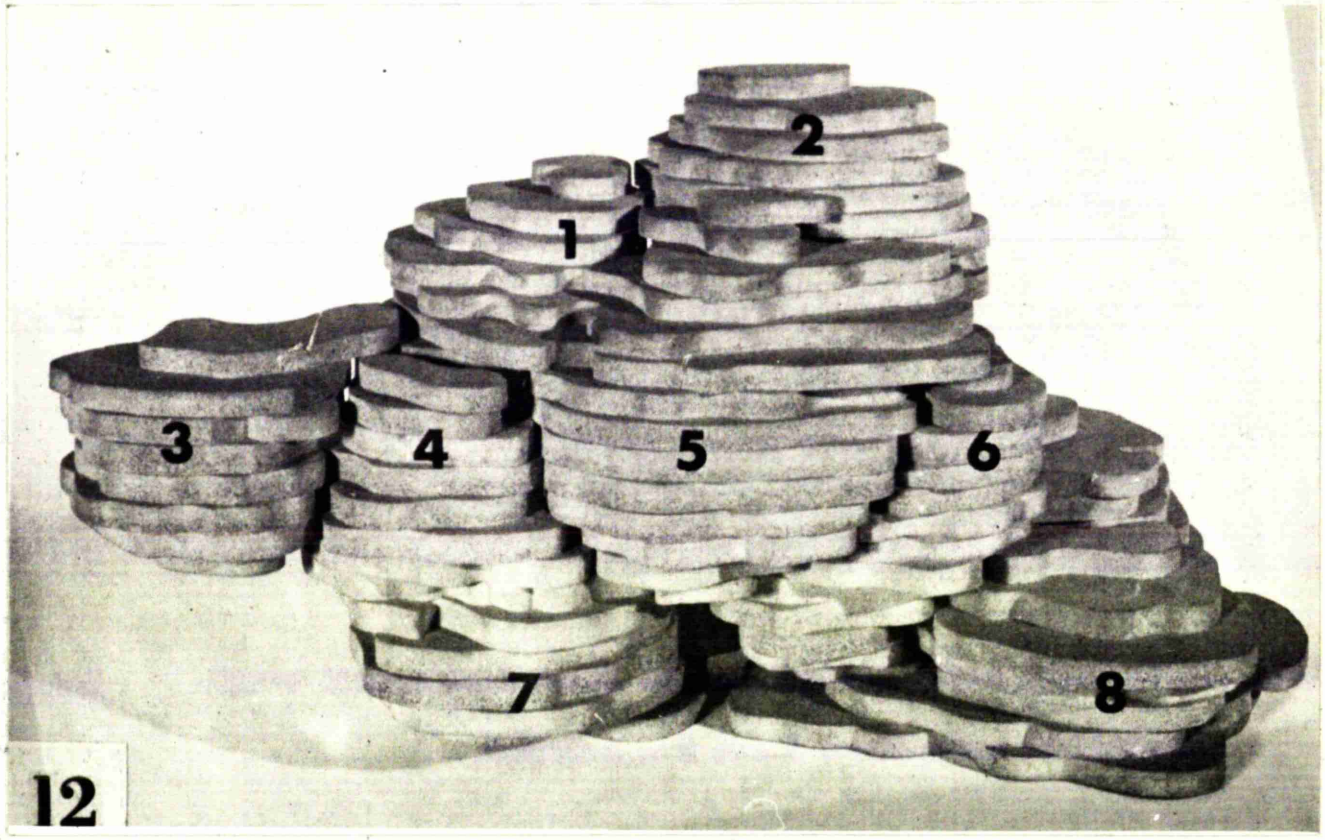


FIG.A-2 Three dimensional model of rat thyroid (from Isler et.al. 1968).

Number 1 - 8 are 8 contiguous follicles.

cells or "C" cells. The whole organ is surrounded by a loose capsule. Arterial blood flows inwards and through the interfollicular spaces, venous return being along a similar path.

Individual follicles range in diameter from 15 - 150 um but most have diameters in the range 20 - 60 um, the smaller being in the central parts of each lobe (Wollman 1965). The follicular cells are cuboidal cells, the average cell in the normal gland being a cube 5 um^3. The mean height of the follicular cells is inversely proportional to the diameter of the colloid lumen so that the smaller the follicle the greater the ratio of inner epithelial area to colloid volume. The basis of this topographical distribution of follicle sizes is not known, but the smallest follicles are in the central parts of the gland. The follicle population is not, however, arranged in a strictly definable geometric pattern. The model built by Isler, Sarkar, Thomson and Tomkin (1968) shows this. (Fig.A-2)

In the average normal adult rat gland colloid occupies about 50 per cent of the gland volume. Colloid is a thyroglobulin ρ homogeneous fluid which is contiguous with the inner walls (apices) of the follicular cells and has a molecular weight of 660,000 - 690,000 (Bloth and Berquist 1968). The apical membrane is about 200 A^o thick and has a series of very short (0.5 um) microvillous extensions. The nucleus of the normal follicular cell is spherical or slightly ovoid, it measures about 3 um^3 and is situated towards the outer end (basal) of the cell. The basal membrane is about

150 A^{o} thick and it is fenestrated by projections from the closely adherent capillaries. The follicular cells are arranged in a pallisade with filamentous connections between cells at the apical and basal ends. The continuous basal membrane is the perifollicular membrane. There do not appear to be other intercellular connexions. Minute capillary shoots lie between the waists of the cells, but these do not reach the colloid cell interface. The follicular cells occupy about 40 per cent of the normal gland volume and since about 50 per cent of the volume is occupied by colloid the remaining 10 per cent is occupied by the interfollicular connective tissue, stromal cells and parafollicular cells (Santler 1957, Hirsch and Munson 1969). In the normal gland follicular cells are about 70 per cent of all cells; of the remainder about 29 per cent are stromal cells and about 1 per cent are parafollicular cells. Stromal cells are spindle shaped and of smaller volume than follicular cells.

The ultramicroscopic character and topography of follicular cell cytoplasm is not different from that of other protein synthesising mammalian cells and all follicular cells have similar features. They have well developed endoplasmic reticula within which are numerous ribosomes. They have Golgi bodies, mitochondria and lysozome bodies. These structures are chiefly, but not exclusively, situated towards the apical end (colloid) of the cell the nucleus lying towards the basal end. Colloid droplets

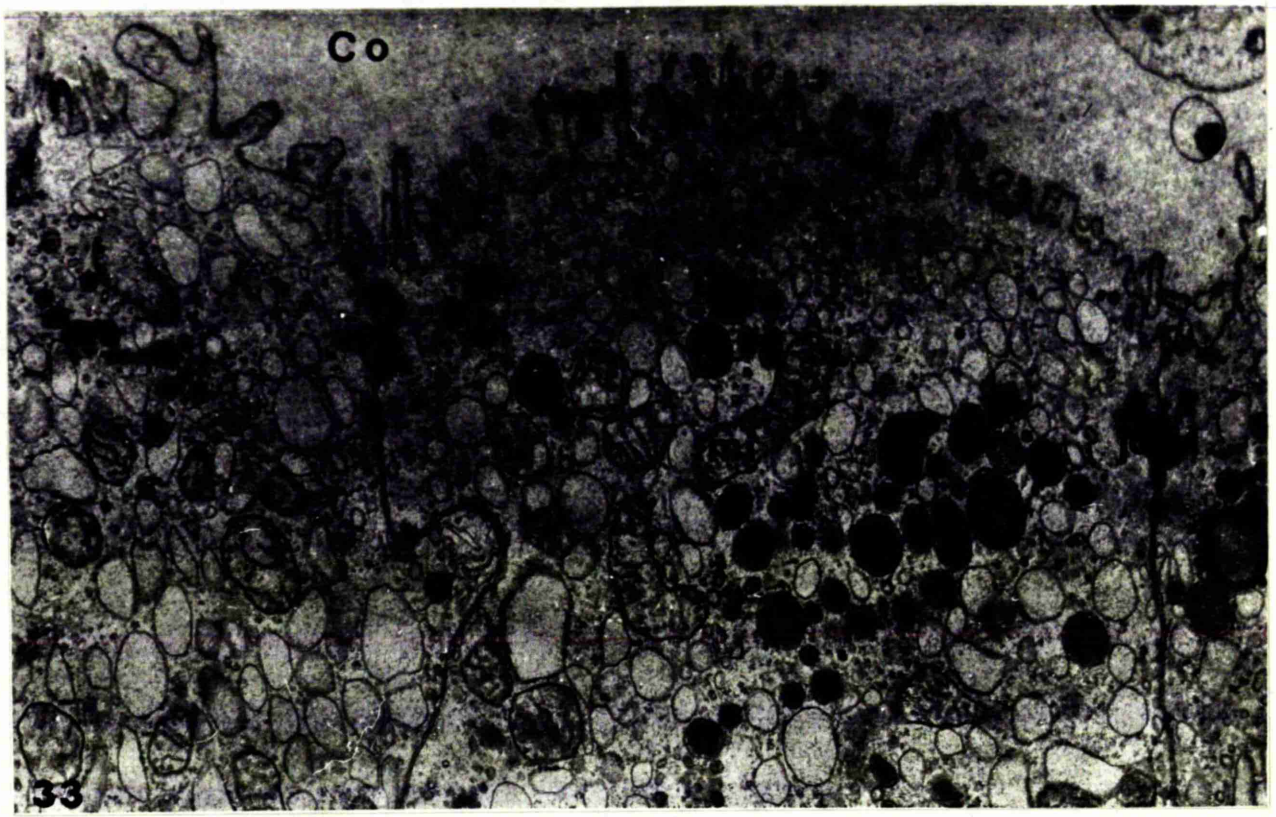


FIG.A-3 Electron Micrograph (x40,000) of untreated thyrotoxic thyroid from Heimann (1966). Co is colloid and apex of cell contains vesicles (ve), mitochondria (mi), dense bodies (db), and junctional complexes (jc).

of various sizes and definability are also seen within follicular cells. The largest and most readily definable colloid droplets are seen at the apical end of the cell near the microvilli. Colloid droplets are colloid thyroglobulin engulfed by the microvillous projections of the apical membrane but some may be new thyroglobulin about to be transferred into the follicular lumen. These aspects are vastly more prominent in active thyroid such as in human thyrotoxicosis. (Fig.A-3)

Structure in Relation to Hormonogenesis.

The fine structural detail of the follicles and follicular cells can, to a large extent, be correlated with what is known of the processes of thyroid hormonogenesis. The follicular cells make, store and secrete, tetra-iodo-thyronine (T4) and tri-iodo-thyronine (T3). The pathways and topography of the cell structures involved in hormone formation, storage, resorption and release in rat and other thyroid have been studied and reviewed by De Groot (1965), Deiss and Peake (1968), Nadler, Young, Leblond and Mitmaker (1964), Seed and Goldberg (1965), Pitt-Rivers and Cavalieri (1964), Edelhoch and Rall (1964), Thomson and Goldberg (1968), Thomson and Bissett (1969), Stein and Gross (1964), Williams and Vickery (1965), Nadler, Leblond and Bogoroch (1964), Nunez, Money and Becker (1966), Wollman (1965), Ekholm and Strandberg (1967) and Seljelid and Nakken (1968). The diagram shown in Fig.A-4 was constructed by myself but is based on a review of the literature cited above.

Topograghy of Hormone Production [Diagramatic]

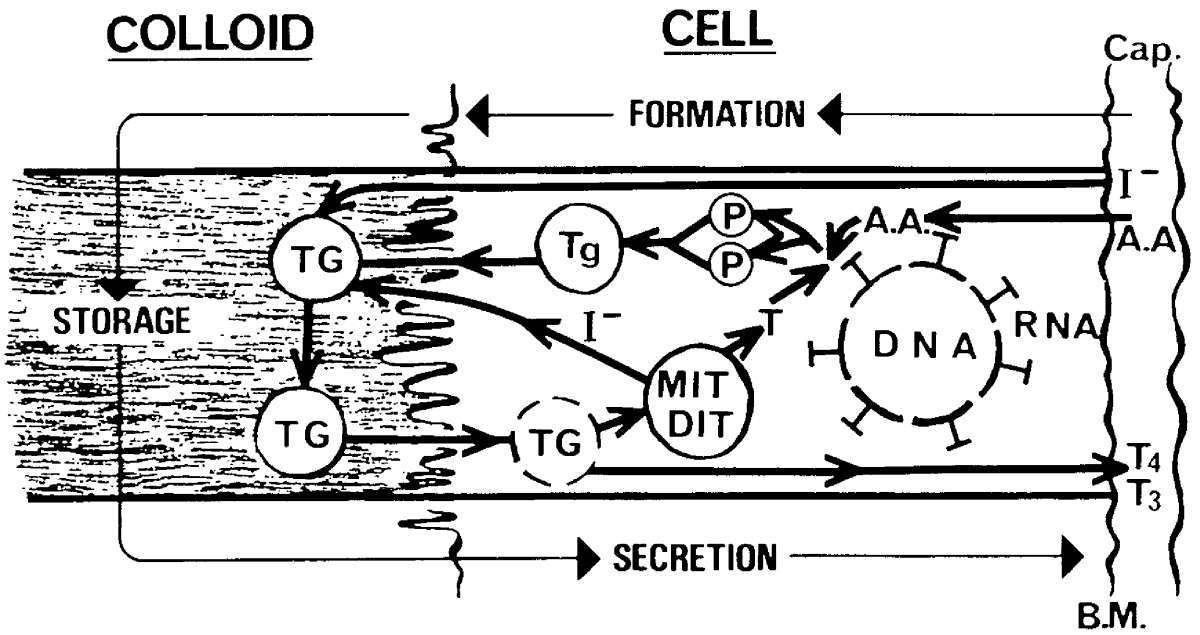


Fig. A-4 Diagram of topography of hormonogenesis in a single follicular cell and based on literature review. See text for explanation of symbols.

Hormone Formation and Storage.

The spheres of follicular cells, and all follicular cells appear to function similarly and simultaneously, remove amino acids (AA) from the arterial capillary and synthesise polypeptide units (P) whose sedimentation coefficient determined by sucrose density ultracentrifugation is 6 Svedberg units (6S proteins). The 6S units appear to link through disulphide bonds (- S - S -) to produce larger polypeptide units (12S proteins). Two 12S units then aggregate through predominantly co-valent linkages to form a dimer, and a carbohydrate component is added. The resulting glycoprotein is new, but immature, thyroglobulin (Tg) which has a sedimentation coefficient of 17S (17S protein). 17S protein is non-iodinated thyroglobulin. The synthetic sequence, amino acid - 6S - 12S - 17S proteins appears to take place in the endoplasmic reticulum proceeding in the general direction cell base across to cell apical margin and away from the nucleus (D.N.A.). Certainly 17S thyroglobulin (Tg) is predominantly located near the apical membrane across which it is transferred into the lumen colloid at its circumference. The latter is contiguous with the apical margin and its microvillous extensions. It is in this position that iodination (I), apparently a special function of apical membrane, takes place but actual hormone (T4 and T3) formation probably occurs throughout the colloid volume. Thus final and definitive hormonogenesis is a function not of the body of the cell, but of the

apical end of the cell and of colloid thyroglobulin. Furthermore iodine accummulation takes place in the colloid and mature colloid thyroglobulin (TG) is a 19S protein.

Iodine Metabolism.

Iodide (I) is removed from the basal capillary and passes immediately through the follicular cell up to and across the apical membrane. The iodide atoms are there oxidised to iodine atoms. Only iodine atoms iodinate thyroglobulin, and it is only tyrosines in the 17S protein which are iodinated. Monoiodotyrosines (M.I.T.) and diiodotyrosines (D.I.T.) are formed simultaneously and not in sequence. At the colloid cell margin, and throughout the colloid, M.I.T. combines with D.I.T. to form tri-iodo-thyronine (T3) and D.I.T. combines with D.I.T. to form tetra-iodo-thyronine (T4). The mechanism of these coupling reactions ismot fully known but iodination and hormone formation confers increased stability on the colloidal thyroglobulin. This mature thyroglobulin (TG) thus contains the thyroid hormones bound in storage; mature colloid thyroglobulin has a sedimentation coefficient of 19S (19S iodo-protein). Definitive hormonogenesis is thus located in the colloid and about 90 per cent of the gland iodine is in colloid only about 10 per cent being within the follicular cells under physiological circumstances.

Hormone Resorption and Secretion.

Colloid thyroglobulin (TG) is taken into the follicular cell and after proteolysis free T4 and free T3 are released and

secreted across the cell and into the basal capillary. The sequence, topographical and biochemical, of colloid resorption, thyroglobulin proteolysis and hormone release appears to be as follows. The apical microvilli surround a droplet of peripheral colloid and, by microphagocytosis (pinocytosis) take it into the body of the cell. Near the apical end of the cell the thyroglobulin droplets fuse with lysosome units and the thyreglobulin drop is fragmented into progressively smaller droplets. These fragments then undergo proteolysis and free T4 and free T3 are released. The freed hormones are then secreted across the basal membrane (BM) and into the basal venous capillary. Proteolysis also releases free M.I.T. and D.I.T., but these are not normally secreted into the basal capillary; the free M.I.T. and D.I.T. have their iodine removed by dehalogenase enzyme. This free iodine is then re-utilised for thyroglobulin iodination at the cell apex and free tyrosine (T) for polypeptide (P) synthesis.

Thyroid Structure and Hormonogenesis in Relation to Current Studies.

The structural architecture of the rat thyroid, the kinetics and locations of protein synthesis, thyroglobulin formation, iodination, hormone storage, resorption and secretion are relevant to the microscopic radiation dosimetry arising from radioactive iodine-131 and iodine-125 and to the location, definition, and measurement of radiation effects on hormonogenesis and on cell proliferation.

Although hormonogenesis is a complex, continuous kinetic process, one of the important facts from the dosimetric point of

view if that 90 per cent of the iodine in the adult rat thyroid is in the colloid thyroglobulin, and only 10 per cent is within the follicular cells. This means that most of the irradiation emissions arising from the decay of radioactive iodine-131 or iodine-125 in the thyroid emerge from the colloid spheres. If these irradiations have ranges which are much greater than the linear dimensions of the follicular cells, or of whole follicles, colloid location is not critical since all parts of the follicular cells, apex, cell body, nucleus and interfollicular stroma are uniformly irradiated and this is true for iodine-131 irradiations. When, however, the irradiations from radioactive iodine in the colloid have ranges less than the length of the follicular cells colloid location is critical because the apical ends of the cells will receive greater doses than their nuclei or basal ends and mutual irradiation of follicles will be less and as will be shown this is true for iodine-125 irradiations. (Section B)

These comments explain why in this thesis a detailed study was made of iodine-131 and iodine-125 dosimetry (Section B) and the effects of their irradiations on hormonogenesis (Section C). The parameters of hormonogenesis studied were follicular cell thyroglobulin synthesis, thyroglobulin iodination, thyroglobulin resorption, thyroglobulin proteolysis and net hormone secretion.

Normal adult rat thyroids were first irradiated <u>in vivo</u> and together with non-irradiated rats (controls) the animals were later given an iodine-deficient diet to increase thyroid protein and new

thyroglobulin synthesis. Thyroglobulin synthesis was studied using pulse labels of tritiated leucine (H3-L). The incorporation of the H3-L into new thyroglobulin was evaluated using total gland incorporation of H3-L and incorporation of H3-L into 19S thyroglobulin, isolated by linear sucrose density gradient ultracentrifugation of the thyroid proteins. The amounts of the different proteins in the gradient fractions was measured spectrophotometrically using sheep thyroglobulin as standard 19S thyroglobulin (Thomson and Goldberg 1968, Thomson and Bissett 1969). In similar studies, thyroglobulin iodination was studied using an iodine-deficient diet and equilibrium labelling of intra-thyroidal iodine with tracer amounts of iodine-125 in drinking water.

These measurements of thyroglobulin synthesis and thyroglobulin iodination represent measurement of hormone formation and storage (Fig.A-4). Complementary studies designed to measure thyroglobulin composition, resorption, proteolysis and hormone release were also conducted after irradiation and described in Section C. After irradiation rats were given a low iodine diet and equilibrium labelling with iodine-125 was established. At the end of this phase the glands were prepared for measurement of free iodide, free M.I.T. and D.I.T. and free T4 and T3 using radiochromatographic techniques; becauseequilibrium labelling was nearly complete the distribution of radioactivities represented the distribution of the non-radioactive iodide and iodinated composition, resorption and proteolytic release of iodinated amino acids and hormones within the follicular cells. Lastly, to obtain an overall index of net

thyroid hormonogenesis, defined as the amounts of T4 and T3 reaching the circulation from the thyroid, the rat blood concentrations of thyroid hormone was measured chemically at the end of each experiment. This was the serum P.B.I.127 concentration.

Structure in Relation to Cell Proliferation.

Very little is known about the structures which are critical to thyroid cell proliferation but the nucleus is thought to be the most important (Moore and Colvin 1968, Speight, Baba and Wilson 1968). In Sections D and E the comparative effects of x-rays and iodine-131 and iodine-125 irradiations on follicular and stromal cell proliferation are described. In Section D the effects of X-irradiation alone on cell proliferation are described in detail. Follicular and stromal cell proliferation was measured after external X-irradiation over a wide range of doses (from 0 to 1000 rads) had been given to normal adult rat thyroid. Cell proliferation was measured sequentially without and with a goitrogenic cell proliferation stimulus (Doniach 1953, 1963, Al-Hindawi and Wilson 1965), the goitrogenic growth stimulus being aqueous methyl thiouracil as drinking fluid for 28 days. This drug stops thyroglobulin iodination and thus also hormone formation storage and secretion. During its continuous administration the rat blood hormone levels fall and the thyroid comes under increasing thyrotrophic hormone stimulation (T.S.H. stimulation). As a consequence, the follicular cells increase in length and

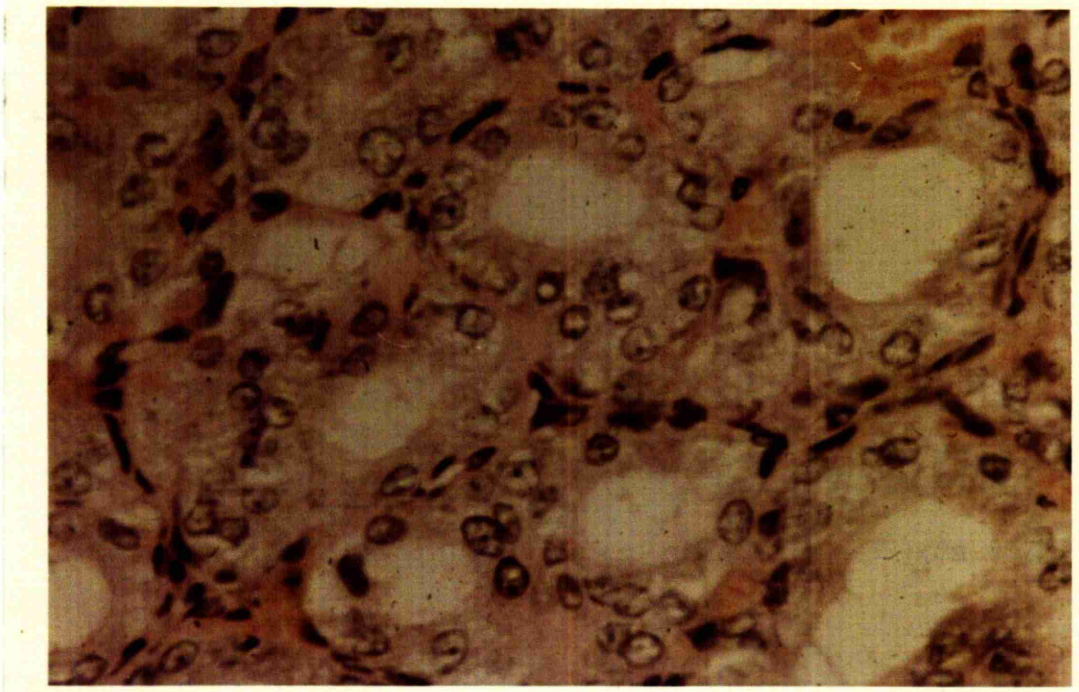


Fig. A-5 Rat thyroid (x400) 14 days after goitrogenic stimulation.

Compare with Fig. A-1.

volume (hypertrophy) and simultaneously multiply (hyperplasia) as confirmed by Philp, Crooks, MacGregor and McIntosh (1969). These changes in follicular cells are accompanied by increased stromal cell proliferation and vascularity, and the luminal colloid volume shrinks (Fig.A-5). The rat cell populations were studied in detail and sequentially after X-irradiation and during the 28 day goitrogenic stimulus and the studies are described in Section D. Measurements included organ weight, total cell density, follicular and stromal cell proportions, chemical D.N.A. and R.N.A. contents of gland and uptake of pulse labels of the D.N.A. precursor tritiated thymidine (H3-T). The latter was studied using nucleic acid extraction and liquid scintillation counting for cell total D.N.A.-H3-T content together with autoradiography for cell labelling indices. This special study using X-irradiation alone and non-irradiated controls provided basic information on the growth characteristics and cell kinetics of the non-irradiated and the uniformly irradiated rat thyroid. Some insight into the possible mechanisms of irradiation interruption of thyroid cell proliferation was also obtained. The studies, however, were most useful in deciding how to use the rat thyroid as a model with which to (specifically) study radiation effects on thyroid cell survival and what data to use to construct radiation-cell survival graphs.

In Section E the model, based on the studies in Section D, was used to measure the relative biological effects (R.B.E.) of

x-rays, iodine-131 and iodine-125 irradiations on rat thyroid cell proliferation and the dose-cell-survival data was interpreted after careful adjustment for factors such as total dose, dose-rate, and quality as calculated in Section B. It was found that the lethality of iodine-125 irradiations on thyroid cells was less than that of X-irradiations or iodine-131 irradiation taking mean thyroid dose as the radiation reference. The difference was explicable on the basis of ultramicroscopic dose distributions in relation to the topography of the cell, and especially the position of the nuclei. The study showed for example that the iodine-125 doses to the follicular cell nuclei were much less than the mean to the gland. The important conclusion stemming from this study is that thyroid follicular cell viability is more dependent on nuclear dose than on mean cell dose even when the inner part of the cell receives the greatest dose and the gland as a whole receives a large dose. The general importance of these studies to our understanding of irradiation effects on normal human thyroid is discussed when appropriate but the dosimetric aspects in relation to cell structure and function of thyrotoxic human thyroid are given special consideration in Section F in relation to the radiation treatment of thyrotoxicosis. Section F therefore includes a review of the problems of iodine-131 therapy for thyrotoxicosis and the rationale design and results of two pilot studies of iodine-125 therapy for thyrotoxicosis are described. In the light of these results the

plan for long term trials of iodine-125 therapy is given.

SECTION B

RADIATION DOSIMETRIES IN RAT THYROID

PRINCIPLES, METHODS AND CALCULATIONS

INTRODUCTION.

In Section A the minute structure of the normal rat thyroid was described. The typical adult whole gland, is two tapering ellipsoids (5mm x 2 mm x 2 mm) and weighs about 20 mg. The parenchymal units are follicles which are spheres of cuboidal cells surrounding cores of thyroglobulin (colloid). The total diameter of follicles (cells plus colloid) varies, the largest follicles being in the periphery of the gland but most have diameters of 20 - 60 um. About 90 per cent of the gland iodine is in the colloid thyroglobulin because iodide trapped by the follicular cells is immediately transferred across the cell up to the apical membrane and incorporated into new thyroglobulin. Although the colloidal thyroglobulin iodine does eventually pass back into the cell during thyroglobulin resorption and proteolysis, the intracellular location of iodine is no more than 10 per cent (Williams and Vickery 1965, Stein and Gross 1964, Nadler, Sarkar and Leblond 1962, Wetzel, Spicer and Wollman 1965, Wollman, Spicer and Burstone 1964). A normal rat thyroid model will be used for discussing dosimetry. In this model selected by myself on the basis of a literature review (Section A) the colloid occupies 50 per cent of the total volume of the gland, the interfollicular space 25 per cent, and only 25 per cent of the volume is occupied by the follicular cells. The latter, however, are 70 per cent of all cells, 30 per cent being interfollicular connective tissue, vascular space and endothelial cells. In the model each follicular

cell is considered to be a cube, (5 um^3) with a nucleus (3 um^3).

In this Section (B) details are given of the irradiation techniques used in all the studies, and of the physical and biological measurements made to determine dosimetry. Special consideration is given to the calculation of the microscopic and ultramicroscopic topographical distribution of absorbed doses using the model and placing emphasis on the differences between external X-irradiation, iodine-131 and iodine-125 irradiations with reference to apical end of follicular cell (hormonogenesis), to cell nucleus (the presumed site of reproductive integrity) and to interfollicular stroma and stromal cells (the nutritional framework).

The techniques used to deliver the irradiations and the method used to calculate mean thyroid doses (rads) will be described first. The calculation of homogeneity and inhomogeneity of dose at the ultramicroscopic level will be described separately.

Delivery and Calculation of Mean Thyroid Absorbed Doses (Rads).

External X-irradiation.

The rats were anaesthetised with a single intraperitoneal injection of 2 - 3 ml of freshly prepared warm sterile 2.5 per cent aqueous tribromethylalcohol (Avertin - Winthrop) Crooks, Greig, MacGregor and McIntosh 1964). Anaesthesia was induced within a few minutes, was not associated with respiratory depression and lasted up to 15 minutes; the mortality was less than 5 per cent.

The anaesthetised rats were placed supine into a deep square perspex jig filled with cotton wool to minimised X-irradiation back scatter. The top was a sliding lid, which could be slotted at different levels above the rat to ensure that the rat neck was slightly compressed. This arrangement ensured that all the rat thyroids were equidistant (0.5 cm) from the X-irradiation collimator whose end was brought down flush on to the transparent lid and into a 2 cm cut out in a sheet of lead. X-irradiation was delivered with a Maxitron Therapy Unit fitted with a special perspex collimator (diameter = 2 cm). The physical factors were: 300 KeV, 20 mA, 23 cms F.S.D., H.V.L. = 0.6 mm.cu. The delivered dose (r) was checked periodically using a Victoreen ionisation ratemeter placed in the perspec jig in the geometric position occupied by the rat thyroids.

As far as could be determined only the thyroid and parathyroid tissues were irradiated and the dose to the pituitary was negligible since the rat's head was slightly flexed to keep the pituitary out of the radiation field. Conversion of delivered dose (r) to absorbed dose (rads) was made using the factor 0.9. This is the factor appropriate to the quality spectrum of the irradiation delivered and given to soft tissue (density = 1G/ml) with minimal back scatter (Meredith and Massey 1968). The x-ray dose rate within the rat thyroid was 190 rads per minute.

Iodine-131 Irradiations.

It is appropriate to first briefly outline the nuclear processes used in the production of commercial iodine-131 (Radiochemical Centre - Amersham, England) and to describe the physical and decay characteristics of the isotope.

Production of Iodine-131.

Iodine-131 is prepared by the thermal neutron irradiation of natural tellurium at a flux of 10^{14} n/cm^2/sec for two weeks. The tellurium used is in the form of the high purity tellurium dioxide and this is sintered at 750-800° before irradiation to remove any volatile impurities (Charlton and Lohman 1969).

The nuclear processes are:-

$$^{130}\text{Te}\ (n,\gamma)\ ^{131}\text{Te} \xrightarrow{\beta^-} {}^{131}\text{I} \qquad \sigma = 0.2\text{b}$$

$$^{130}\text{Te}\ (n,\gamma)\ ^{131m}\text{Te} \xrightarrow{\beta^-} {}^{131}\text{I} \qquad \sigma = 0.03\text{b}$$

However, at the same time, long-lived iodine isotopes are also produced by the reactions:-

$$^{126}\text{Te}\ (n,\gamma)\ ^{127}\text{Te} \xrightarrow{\beta^-} {}^{127}\text{I (stable)} \qquad \sigma = 0.9\text{b}$$

$$^{128}\text{Te}\ (n,\gamma)\ ^{129}\text{Te} \xrightarrow{\beta^-} {}^{129}\text{I}\ (t_{\frac{1}{2}} = 1.6 \times 10^7\ \text{yr}) \qquad \sigma = 0.14\text{b}$$

Because of these, the "carrier-free" concentration of 1.24×10^5 Ci/g iodine cannot be reached but specific activities obtained usually lie within the range $0.5 - 2 \times 10^4$ Ci/g.

The irradiated target is heated to 750-800°C to distil iodine from tellurium dioxide and the iodine is dissolved in sodium

hydroxide solution. The solution of sodium iodide-131 so produced is, after appropriate treatment, used for the preparation of iodine-labelled compounds, for protein iodination and for the treatment of thyroid disorders (IBS1P, IBS2P - Radiochemical Manual). This latter material is treated with excess sodium thiosulphate to prevent oxidation of the iodide to iodate or periodate and was the preparation used in the current experiments.

Physical and Decay Characteristics of Iodine-131.

The physical and decay characteristics of iodine-131 (I_{53}^{131}) have been studied in detail (Graeffe and Walters 1967). Seven different modes of beta decay have been confirmed, each leading to the formation of xenon-131 Xe_{54}^{131}. However, 90 per cent of the beta transitions of iodine-131 involve the transfer of 606 KeV energy to an electron neutrino pair and leave the daughter xenon nucleus in the 365 KeV excited state. In an 806 KeV transition 0.8 per cent of the iodine-131 decays to a metastable state of 131 Xe that has a half-life of 12 days. The latter has a high probability of electron conversion decay to the ground state. Each atom of iodine-131 can decay only once, by one of the seven processes. The percentages stated in Table B-1 are the "branching ratios" and give the odds in which route is likely to be followed. However, the predominant particle spectrum must have E_{β}^{Max} = 608 KeV and the greatest number of gamma ray protons will have E^{Max} = 365 KeV. In the calculations of mean absorbed doses within rat thyroid from iodine-131 more than 90 per cent of which is B-irradiation an $\bar{E}_{\beta}$

Table B-1

Emissions Arising From Decay of Iodine-131

(Physical Half-Life = 8.03 days)

Photon Emissions.

Energy (KeV).	Abundance (Per 100 I-131 Decays)	Order of Range in Tissue
80	2.20	> 100 um
163	0.70	> 150 um
284	5.30	> 500 um
364	80.00	"
637	9.00	"
722	3.00	"

Electron Emissions.

565	0.02	cms.
606	90.00	"
619	0.04	"
806	0.80	"

value of 187 KeV was used. As is shown in Table B-1 the ranges in tissue of all the β radiations are in excess of 100 um and the ranges of all the γ radiations are of the order of cms. These comments are relevant to the discussion on homogeneity and inhomogeneity of dose distribution within this tissue (see below).

Iodine-131 Administration and Calculation of Mean Absorbed Dose (Rads).

Radioactive iodine-131 (IBS2P Radiochemical Centre, Amersham) was made up in sterile saline and administered intraperitoneally to rats in volumes of 0.5 - 2 ml and in uci measures. The mean β radiation absorbed doses (rads) within the whole rat thyroid were calculated using a basic β dose formula with adjustments for biological factors such as loss of irradiation from the periphery of the gland and shortening of the effective half-lives following very large doses of irradiation. The mean β iodine-131 dose throughout the rat thyroid was calculated from the standard formula for infinite β dosimetry:

$$D_\beta = 73.8\ \bar{E}_\beta\ C_o T_{eff}\ \text{(rads)}$$

where $\bar{E}$ (MeV) is the local β energy deposited per disintegration, C_o (uCi/g) is the isotope concentration at time of maximum uptake and T_{eff} (days) is the effective half-life, (Loevinger, Holt and Hine 1958). For iodine-131 $\bar{E}\beta$ is 0.187 MeV.

The factor C_o, and the factor T_e were measured directly. At each sacrifice mean thyroid weight and mean thyroid content of

iodine-131 was made. When thyroid content of radioactive iodine-131 was to be measured the thyroids were disintegrated in 3 ml of 2N NaOH and the radioactivity determined by well counting; comparison with standards gave the uci content and mean thyroid weight was used to calculate C_o (uCi/g). In the experiments to be described it was found that the maximum uptake occurred within 24 hours of the injection of the radioactive iodine and the average T_e was not less than 4 days; because the uptake phase was rapid compared to the loss phase, dose calculations were based on the latter only as discussed by Loevinger, Holt and Hine (1958). In the case of normal rat thyroid and iodine-131 β irradiation there is a surface loss of energy because the size of the lobe (5 mm x 2 mm x 2 mm) is not very large compared to the mean and maximum ranges of the β rays (500 and 2000 um respectively). The mean absorbed iodine-131 β dose calculated by the formula was therefore reduced by 20 per cent to allow for surface loss of β energy (Loevinger, Holt and Hine 1958).

The mean absorbed doses to rat thyroid from iodine-131 gamma radiations are less than 5 per cent of the total. I, therefore, chose to neglect this irradiation from iodine-131.

For the largest administered amounts of iodine-131 an allowance was made for a reduction in thyroid biological half-life (B.H.L.) of up to 15 per cent for the most highly irradiated glands as recommended by Maloof, Dobyns and Vickery (1952). The mean

absorbed doses in the glands were finally calculated on the basis of all these considerations.

Iodine-125 Irradiations.

It is appropriate to briefly describe the nuclear processes involved in the commercial production of iodine-125 (Radiochemical Centre, Amersham) and it is essential to give a full description of the physical and decay characteristics of this relatively new and complex isotope, whose potential uses in medicine were first described by Myers and Vanderleeden (1960) and by Harper, Siemens, Lathrop and Endlich (1963) and further examined in tracer studies by Ben-Porath, Hochman and Gross (1966) and by Myers (1965).

Production of Iodine-125.

Iodine-125 is produced by the thermal neutron irradiation of natural xenon gas at a pressure of 5000 psi, according to the neutron reaction:

$$^{124}Xe\ (n,\gamma)\ ^{125}Xe \xrightarrow{E.C} {}^{125}I$$

Further irradiation of the iodine ^{125}I leads to the production of iodine-126 ($^{125}I\ (n,\gamma)\ ^{126}I$). The iodine-126 may be limited by a loop process, whereby the iodine is removed continuously preventing further neutron interaction. By the batch process, the iodine-126 is usually less than 1 per cent of the iodine-125 and this percentage decreases still further with time. Iodine is separated from xenon by successive oxidation reduction processes and finally made up as a neutral solution of

sodium iodide-125. The solutions which were used in the current studies were made up in N/50 sodium thiosulphate, which serves as a reducing agent. The specific activity of the iodide-125, so produced, was between 4 and 17 mCi/mg compared with the maximum theoretical specific activity of 17.4 mCi/mg (Charlton and Lohman 1969).

Physical and Decay Characteristics of Iodine-125

The physical and decay characteristics of iodine-125 have been subject to much scrutiny and experiment. The author himself has assisted in a detailed examination of theemissions arising from iodine-125 and their relevance to the dosimetry of iodine-125 in the rat and human thyroid (Gillespie, Orr, Greig 1970). The account given by the latter authors is summarised here.

Iodine-125 decays to an excited state of 125_{Te}, 35.5 KeV above the ground state solely by electron capture; 82.2% of the K-, 14.4% from the L_1-, 0.4% from the L_{11}- and 3% from the M- shells (Wapstra, Nijgh and Van Lieshout, 1959). The γ radiation (mainly magnetic dipole) emitted in the transition to the ground state of ^{125}Te is highly converted; 78% in the K-, 11% in the L_1-, 1% in the L_{11}- and 3% in the M- shells; these abundances being interpolated from theoretical conversion coefficients calculated by Sliv and Bland (1961) and tabulated by Lederer, Hollander and Perlman (1967).

Vacancies created in the orbital electron structure of tellurium, following electron capture and internal conversion, are

TABLE I-2

Radiations Arising From the Decay of Iodine-125.

(Physical Half-Life = 60 days)

Type	Energy Kev	Abundance (per 100 ^{125}I decays)	Mean Energy/disintegration Kev
To γ-rays	35.5	7	2.5
To K^1_{32} X-rays	31.7	4.5	1.4
To K^2_{31} X-rays	31.0	20.1	6.2
To $K_{\alpha 2}$ X-rays	27.2	38.0	10.4
To $K_{\alpha 1}$ X-rays	27.47	74.7	20.3
To L X-rays	3.7	8.3	3.1
		Total	41.9

Electron Emissions

To K-conversion	34.5	4.0	1.4
To L_2-conversion	30.9	1.0	0.3
To L_1^2-conversion	30.5	10.0	3.1
To KLL Auger	29.0	2.3	0.7
To KLL Auger	26.3	6.0	1.6
To KLL Auger	22.7	13.9	3.2
To K-conversion	3.7	78.0	2.9
To LMM Auger	3.0	165.4	5.0
To LLM Auger	0.4	395.0	1.4
To KMN Coster-Kronig	0.3	355.0	1.1
To LLM Coster-Kronig	0.2	29.0	0.1
		Total	28.8

filled in transitions involving the emission either of x-rays or of electrons arising from Auger and Coster-Kronig transitions. The K-shell fluorescent yield of ^{125}Te (w_k = 0.86) is well established but yields for L-subshells and higher order shells are not. Data of Fink, Jopson, Hans Mark and Swift (1966) suggests that the L_1-, L_{11}- and L_{111}- fluorescent yields are 0.03, 0.06 and 0.04 respectively. M-shell fluorescent yields have been measured only for elements of high Z (atomic number); the values for tellurium will be small, certainly less than 3%. Relative intensities of the components of the KLL Auger complex, and ratios of KLY to KLL and KXY to KLL (where X and Y are higher order shells), transition probabilities can be obtained by interpolation of data presented by Wapstra et al. (1959).

Probabilities of L-subshell Coster-Kronig transitions for ^{125}I are givenby Fink et al. (1966). Table B-2 lists the principal X- and gamma-ray and electron emissions, their relative abundances and mean total energies per disintegration, calculated using the above data.

Each Auger or Coster-Kronig transition increases the net ion charge by one, alters the binding energies of outer electrons by amounts which have not yet been reported and thereby introduces uncertainties in the energies of radiated electrons. For this reason and also because Auger and Coster-Kronig transition probabilities for outer shells are not known, the energies of very low energy (0.5 KeV) electron emissions are uncertain. X-ray

yields from very low energy transitions are so low that they can be neglected. The range of 0.5 KeV electrons in water is 0.015 um, and at this level the important histological and biochemical details of the thyroid that affect the microdose distribution are the parts of follicular cells synthesising hormone next to the colloid (see Fig.A-4). It is thus important to know the total mean energy/disintegration due to the very low energy electrons. This value can be calculated by subtracting the sum of the energies due to known electron and electromagnetic radiations from the total average energy emitted in the decay of ^{125}I. The latter is 62.43 KeV; 26.93 KeV due to the transition to $^{125}Te^*$ and 34.5 KeV due to the decay of $^{125}Te^*$ to the ground state. In Table B-2 the total energy of the emissions is 62.7 KeV so that even if the energies and abundances of some of the very low energy radiations prove to be in error, their total energy is accurate.

Iodine-125 Administration and Calculation of Mean Absorbed Dose (Rads)

Radioactive iodine-125 (I.M.S.4 - Radiochemical Centre Amersham) was made up in sterile saline and administered intraperitoneally in volumes of 0.5 - 2 ml and in uci measures. The mean electronic absorbed dose throughout and within the rat thyroid was determined as described under iodine-131 irradiation and using the β-ray dose formula given, with adjustments when appropriate.

In calculating the mean absorbed radiation doses from iodine-125 recent physical data on this isotope were used (Smith 1966, Feige and Gross 1968 and Gillespie, Orr and Greig 1970).

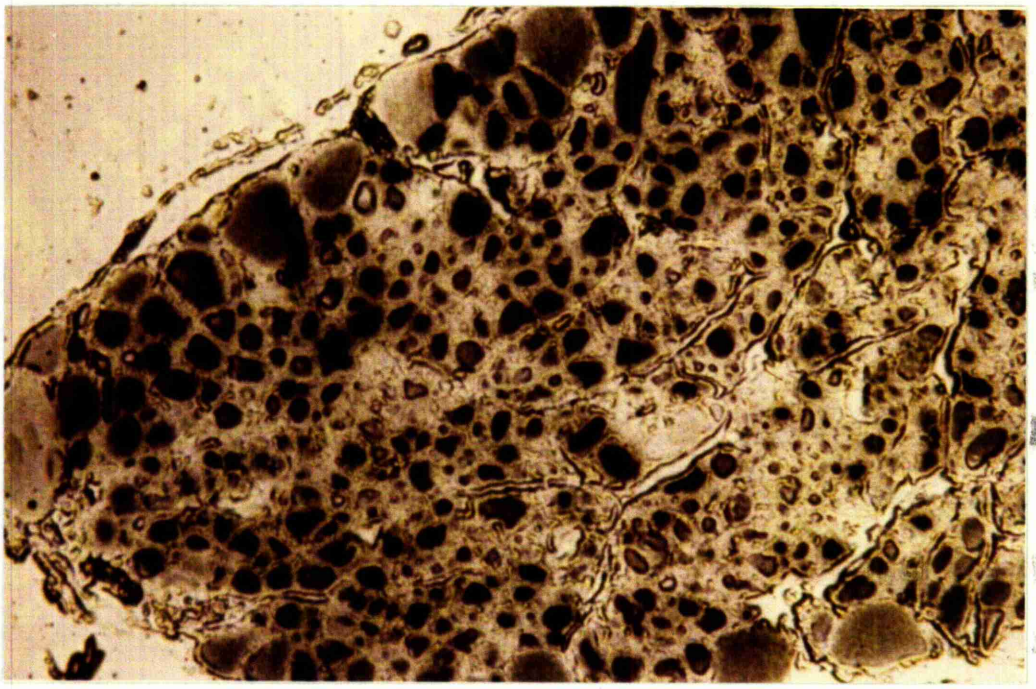
$\bar{E}_\beta$ per disintegration was 0.0205 MeV but no electron surface loss was allowed for because the ranges of nearly all the iodine-125 electrons are very much shorter than the dimensions of one thyroid lobe (see below). A contribution of 0.0034 MeV per disintegration was, however, added for the x-ray emissions from iodine-125 in a tissue with the dimensions of one thyroid lobe. Thus, in contrast to iodine-131 irradiation, iodine-125 irradiation in normal rat thyroid does not require adjustment for surface loss of electronic dose but a contribution of about 15 per cent of the iodine-125 dose is due X-irradiation.

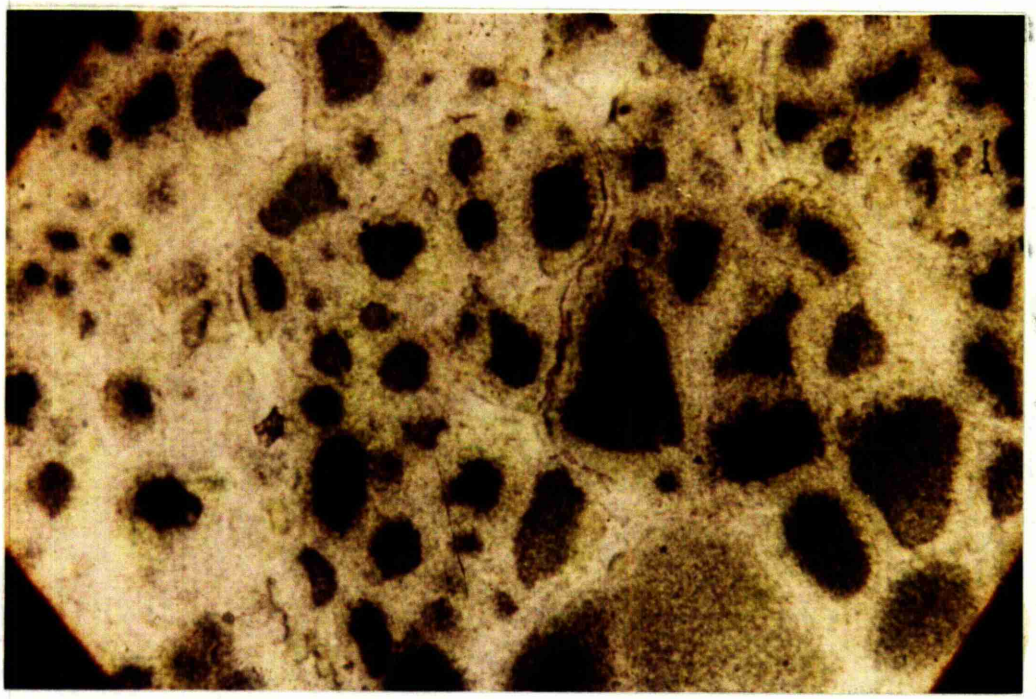
However, as for the largest doses of iodine-131 (to be specified when experiments are described) an allowance was made for shortening of thyroid B.H.L. after the largest doses of iodine-125 (to be specified when experiments are described). For the amounts cited the B.H.L. was reduced by 15 per cent in the final calculation of mean thyroid absorbed doses. Likewise the iodine-125 doses arising from the thyroid uptake phase (18 to 24 hours) were neglected since the T_{eff} for iodine-125 was always in excess of 7 days so that the duration of the uptake phase was much shorter than the duration of the loss phase.

It is now appropriate to turn to a description of homogeneity and inhomogeneity of microscopic and ultramicroscopic dosage distribution arising from external X-irradiation, iodine-131 and iodine-125 irradiation respectively.

Homogeneity and Inhomogeneity of Microscopic Dose Distribution. External X-irradiation.

The X-irradiation characteristics used, and the smallness of the rat thyroid as a whole (depth below skin is less than 0.5 cm) ensured that the absorbed radiation doses were given at a high and steady rate and homogeneously distributed throughout the whole organ. This applies whether distribution is defined as macroscopic, microscopic or ultramicroscopic dimensions. The ionisation "fields", which ultimately determine types and degrees of radiobiological effects, arise at random and homogeneously through the gland during external X-irradiation. This means that, statistically, all molecular units were equally likely to get the same dose at equal dose rates. It can, therefore, be stated that in the externally X-irradiated rat thyroids, the interfollicular stroma, the stromal cells, the parafollicular cells, the follicular cells and the colloid absorbed equal doses (rads). Furthermore, because the secondary ionisation fields have dimensions in Angstoms (A^{o}), the various ultramicroscopic structures inside each cell also receive the same dose which is equal to the average to the gland. For example, within one follicular cell the doses to the nucleus are the same as the doses to the basement membrane, to the basal capillary, to the endoplasmic reticulum, to the Golgi body, to the apical membrane and to the lysosomes in the cell. In structural or biochemical terms this means that the doses to the part(s) of the cells presumptively controlling cell replication were equal to the doses to the parts of the cells involved in





Figs. B-1 - 2. Autoradiographs (x75 and x 200) of rat thyroid 6 hours after I-131. I-131 is mainly in colloid.

hormonogenesis. Furthermore, each structure in all of the cells irrespective of location in the gland received the same doses. These are the reasons why external X-irradiation was used as the radiation control.

Iodine-131 Irradiations.

As described above, 90 per cent of iodine in the average model rat thyroid is in the colloid and 10 per cent is in the follicular cells (Figs.B-1 and B-2). In the model rat thyroid (see Introduction) colloid occupies 50 per cent of the gland volume and 25 per cent is occupied by the interfollicular space and only the remaining 25 per cent of the volume is follicular cells.

In summary of the decay of iodine-131 and the frequencies of the energies an index of their range in tissue (density 1 G/ml) is shown in Table B-1. In the rat thyroid the γ-irradiation, a negligible proportion of the total radiation (less than 5 per cent) and has ranges far greater than the largest axis of one thyroid lobe. The very large majority of the β rays (95 per cent of irradiation) have ranges well in excess of 100 um; the average range is 500 um with a maximum of 2000 um. It can, therefore, be assumed that the rad doses deposited by iodine-131 gamma rays are homogeneous throughout the thyroid at macro, micro and ultramicroscopic dimensions.

The β rays from iodine-131, however, with an average range of 500 um travel within the minimum dimensions of one thyroid lobe (2000 um); it follows that the β-doses to a narrow outer rim of rat thyroid are about half the average to the whole gland. The

volume of this rim is, however, a small portion of the total mass of the gland (about 10 per cent of volume). Nevertheless, it represents a degree of inhomogeneity of dose distribution at the macroscopic level. At the microscopic or ultramicroscopic level, however, and irrespective of the follicular position in the gland the ranges of β rays from iodine-131 are such that the radiations arising from the colloid within even the largest follicle will always be deposited well outside that follicle across interfollicular tissue and into several contiguous follicles. Although the diameters of follicles do vary most are between 20 and 60 um and it has been shown that the smaller follicles compensate for low colloid volume by greater iodine accumulation per unit time (Wollman 1965). As a consequence, the total radiations emitted from iodine-131 in smaller follicles tends to equal that from larger follicles. Thus, irrespective of follicle size mutual irradiation is homogeneous throughout the follicular population and at all microscopic dimensions. As a result the likeliehood doses to all parts, follicular cells, stromal cells, capillaries and the doses to all parts of each cell are those of the average to the gland.

These comments apply whether or not account is taken of linear energy transfer (L.E.T.). The ionisation paths from iodine-131 β-irradiation are not as homogeneous as those arising from external x-rays. As a generalisation the ionisation is relatively sparse along the first part of the β ray tracks, even along the second part and highest as the β particles terminate. Since, however, average track lengths are much longer than the diameters of the largest

follicles (200 um), the random distribution of ionisation fields will be homogeneous and unrelated to any microscopic or biochemical structure in the thyroid.

It may, therefore, be concluded that the irradiation doses deposited within the normal rat thyroid from iodine-131 are homogeneously distributed apart from a small peripheral rim of tissue of about 10 per cent gland volume. Whatever the location of a follicle in the thyroid, the microscopic and ultramicroscopic doses to groups of contiguous follicles equals that to interfollicular stroma and to the mean dose to the gland. In addition the doses to follicular cell nuclei (presumably controlling cell division) are the same as those to the body of the cell and to the apical margin (involved in hormonogenesis.)

Unlike the irradiations from external x-rays and from iodine-131, those from iodine-125 in the rat thyroid are not deposited homogeneously either microscopically or ultramicroscopically. The physical and decay characteristics of iodine-125 (Table B-2), the colloid location of 90 per cent of the radioactive iodine (Figs B1-2), the arrangement and dimensions of the spheres of follicular cells (Fig. A-1) and the biochemical topography of the follicular cell itself (Fig A-4) all combine to assume critical importance in determining a characteristically inhomogeneous dose in the thyroid and across each follicular cell.

Physical and Decay Characteristics of Iodine-125 Relevant to Inhomogeneity of Microscopic Dose.

The basic iodine-125 decay data is given in Table B-2 but a large number of tedious calculations were necessary to obtain the predicted photon (x and γ rays) and electron dose distributions within the rat thyroid. Photon irradiation is discussed first then electron irradiation. Finally calculation of total (photon and electronic) dose distribution within the model rat thyroid described above is possible.

It is best to consider the distribution of photon doses first and then to consider the distribution of electronic doses and finally to add the two together when the total dose distribution in the model rat thyroid is given. It is also helpful to the reader to summarise the relative abundances and ranges in tissue of electrons and photons arising from iodine-125. This is given in Table B-3 which is derived from Table B-2 and the calculations now to be described and also considered by Feige and Gross (1968) and Gross, Ben-Porath, Rosin and Bloch (1968).

X- and gamma-radiations Dose Distributions.

A large proportion (68 per cent) of the total energy released in the disintegration of iodine-125 is, on average, in the form of low energy photons. However, as discussed above, owing mainly to the small size of the rat thyroid only a small fraction of this photon energy is actually absorbed in the rat gland although almost all of the 3.1 KeV/disintegration

TABLE B-3

Principal Emissions from Iodine-125 and Approximate Ranges in Tissue

No. per 100 disintegs.	KeV	Mean KeV per disinteg.	Approx tissue range um
X- and γ-rays (Photons)			
23	3.8	0.2	100
112	27.4	30.6	30×10^3
24	31.1	7.4	50×10^3
7.3	35.5	2.5	70×10^3
Electrons			
376	0.65	2.4	0.02
78	3.70	2.9	0.30
156	4.00	6.2	0.40
15.4	23.00	3.5	12.00
8.8	26.50	1.8	15.00
12.0	31.00	3.7	22.00
3.7	35.00	1.3	26.00

attributable to 3.7 KeV Te L X-rays (Table B-2) will be absorbed since the half-value thickness in tissue of 3.7 KeV photons is 110 um and the shortest axis of one rat thyroid lobe is 2000 um. In the range 24.5 KeV to 35.5 KeV, however, which includes the major photon emissions the energy deposited locally is very low (less than 3 KeV), the remainder being deposited diffusely. Thus, as discussed previously the proportion of the mean absorbed dose from iodine-125 in the rat thyroid due to X-irradiation is only of the order of 15 per cent. The X-irradiation dose which is absorbed and not lost is low energy but it's distribution is homogeneous throughout the gland, because the ranges in soft tissue are of the order of cms and this is vast compared to the dimensions of the rat thyroid. (This is why only about 15 per cent of total energy deposited in the thyroid is photon, most photon radiation being deposited outside the small tissue). The remaining 85 per cent of the absorbed dose is electronic irradiation.

Electronic Irradiation.

A number of tedious calculations were required before the dose distribution in the thyroid could be determined.

Energy Loss of Electrons from a Point Source.

For each of the principal electron emissions of iodine-125 listed in TableSB-2 and B-3, energy loss as a function of distance from a point source was calculated from data presented by Bacq and Alexander (1955). The latter give energy loss per distance as a function of electron energy. This data is shown

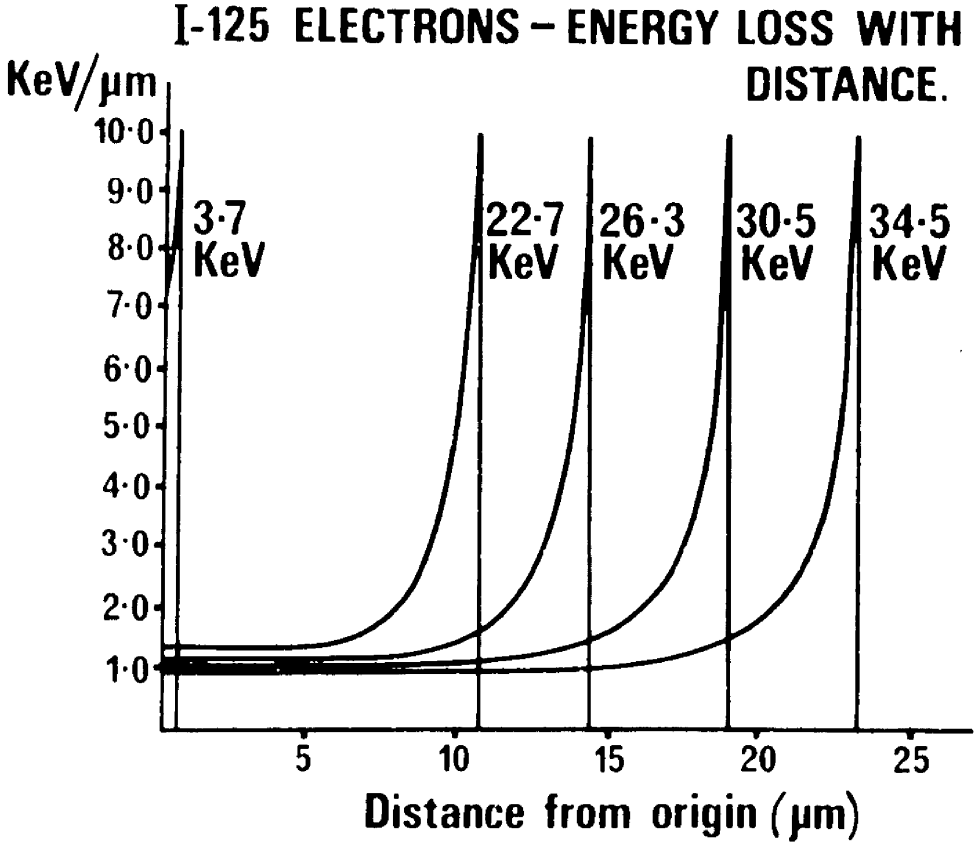


Fig. B-3 Electron energy losses in tissue as function of distance from point source of iodine-125.

in Fig. B-3 which demonstrates that much of the energy is deposited within 15 um of the source and all of it within 25 um of the source. This basic information was expanded to calculate the distribution of electronic radiation doses (rads) arising from iodine-125 in various geometrical sources.

Absorbed Electronic Doses from Iodine-125 in Plane Sources.

The electronic absorbed dose-rate in rads per day arising at different distances from planes containing 1 mci iodine-125 per gram are shown in Fig. B-4. This data was obtained by first calculating the relative dose-rate distributions due to extended uniform plane sources of iodine-125 and arising from each of the principal electronic emissions. Thereafter using simple mathematics the corresponding dose-rate distributions inside and outside semi-infinite sources, and plane sources of various finite thicknesses (1 μ to 8 μ) were determined for each of the principal components. Finally, by weighting each of the various distributions by the abundance of the component (Table B2 - B3) the true electron dose-rate distributions from the sources were determined. The data shown in Fig. B-4 demonstrates that with each plane the dose-rate falls rapidly within the first few um from the surface and in each instance has reached zero at distances greater than 16 um from the surface. The fall in dose-rate from the surface is, as expected, most remarkable when the iodine-125 is in the form of a 1 μ thick plane.

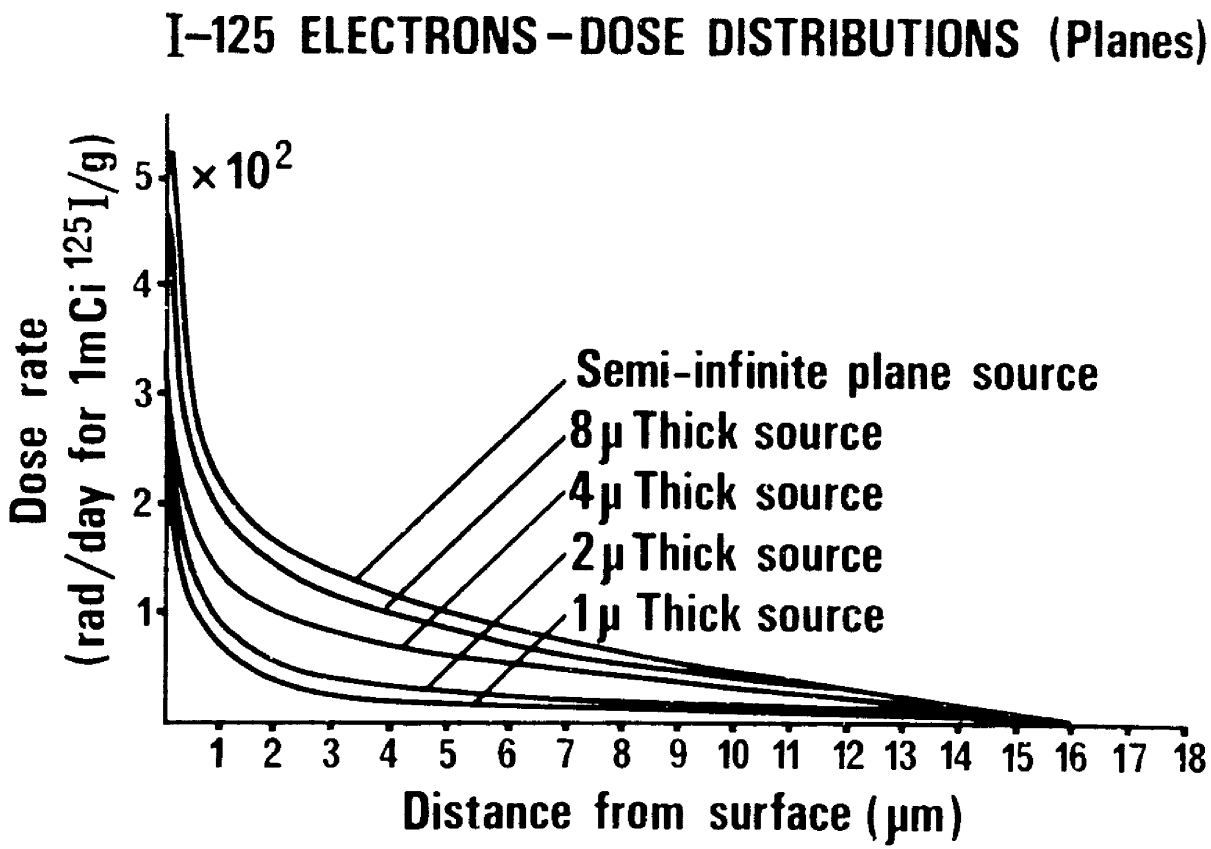


Fig. B-4. Electron dose-rate distributions outside extended uniform plane sources of iodine-125 (1 mci per gram).

Absorbed Electronic Doses from Iodine-125 in Spherical Sources.

It was next possible to calculate using simple mathematics the electronic absorbed radiation dose-rates (rads per day) arising from iodine-125 in spheres. This was done for a sphere of diameter 35 um and for a sphere of diameter 70 um each containing iodine-125 in a concentration of 1 mCi per gram. The electronic dose-rate distribution from an infinite plane source was also calculated. This information is shown in Fig. B-5. The main point is the steep fall in dose-rate across the boundary (0). The fall is such that within 4 um of the surface of the infinite plane source or the spheres the dose-rate is several fold less than it is on the surface (0 um). It is also important to note that the character of the dose-rate graph is very similar for each of the three geometries. The dimensions of the two spheres, 35 um and 70 um respectively were chosen because these are the dimensions of average colloid spheres in normal rat thyroid. Furthermore, all the data shown in Figs. B3 - 5 were calculated on the basis of a source material of density of 1 gram per cm3. This is the density of soft tissue. It is now possible to provide an index of the dose distribution from iodine-125 in the rat thyroid and with respect to photon and electron irradiation and in relation to the micro anatomy.

Absorbed Doses (Electronic and Photon) from Iodine-125 in Rat Thyroid.

The average model rat thyroid has colloid spheres of mean

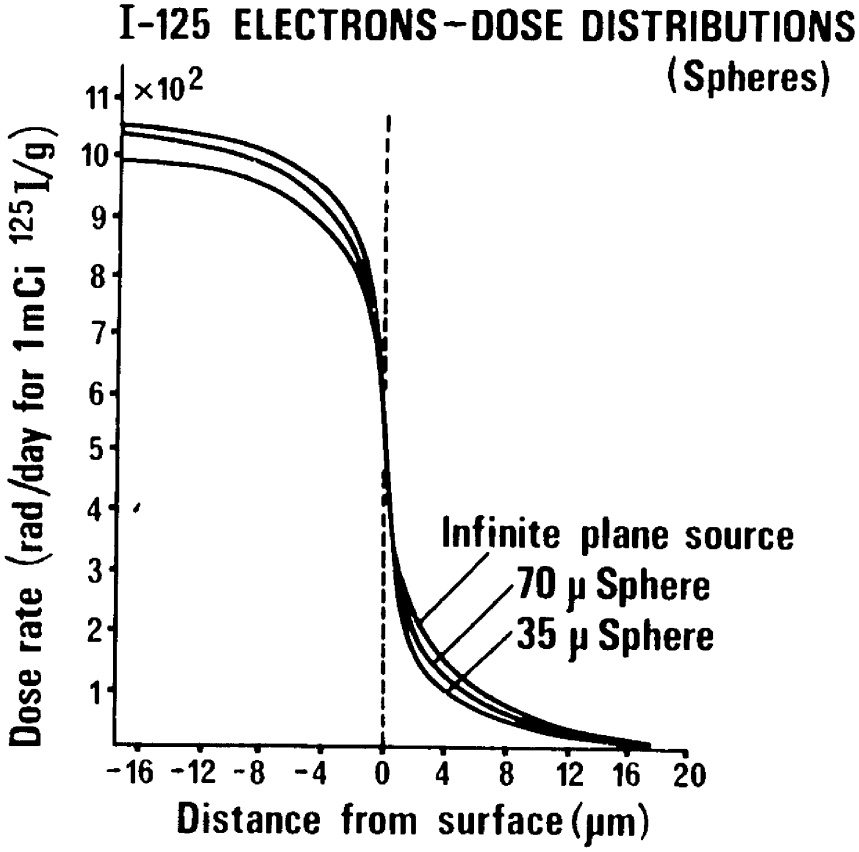


Fig. B-6 Electron Dose-rate distributions due to two uniform spherical sources and an infinite plane source of iodine-125 (1 mCi per gram).

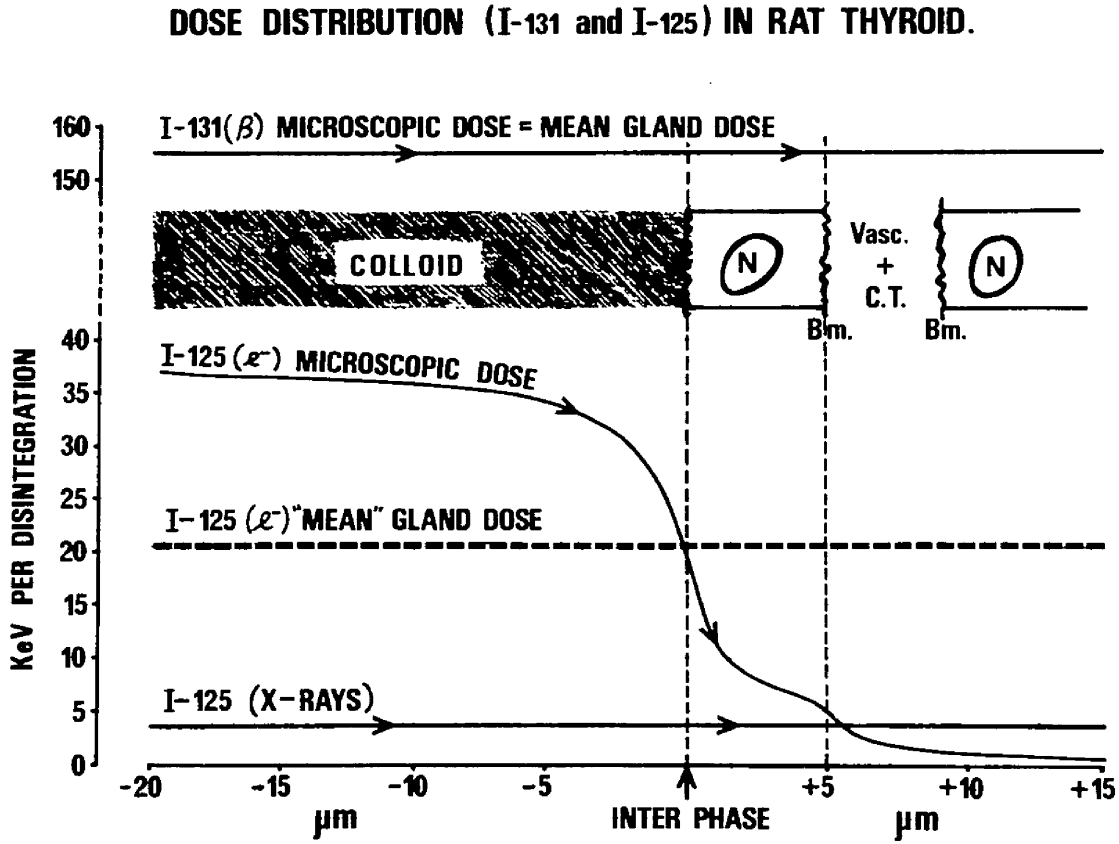


Fig. B-6. Calculated dose (KeV) distribution in segment of rat follicle arising from iodine-131 and iodine-125 (90 per cent in colloid) $\mathscr{E}$ is electron dose, N is nucleus, Bm is basement membrane, vasc.+ C.T. is vascular plus connective tissue.

diameter (about 40 um) and the colloid occupies 50 per cent of the gland volume and contains 90 per cent of the iodine-125; 25 per cent of the remaining volume is follicular cells (surrounding the colloid) and 25 per cent is interfollicular tissue. The remaining 10 per cent of gland iodine-125 is in the follicular cells (Section A). Thus, if the physical data shown in Figs. B 3 - 5 and in particular that in Fig. B-5 are extrapolated to the average rat follicle an index can be obtained of the dose distributions arising from iodine-125 in the colloid and absorbed by the surrounding follicular cells and tissue constituents. A typical segment of a rat thyroid follicle is shown in Fig. B-6 and the relative microscopic dose distributions arising from iodine-125 have been calculated. The data for an equivalent concentration of iodine-131 (uci per gram) is included for comparison. Fig. B-6 shows that the mean dose rates from iodine-131 are nearly 8 times those from iodine-125 in the same concentration in a follicle whose colloid radius is 20 um and whose follicular cells are 5 um^3 cubes. The Fig. B-6 also shows that whereas all the dose from iodine-131 is homogeneously distributed across, through and beyond the follicular cell and into interfollicular stroma only the x-ray component of iodine- 125 (about 15 per cent of the "mean" dose) is homogeneously distributed. The electronic dose (about 85 per cent of the "mean" dose) is very inhomogeneously distributed. The ranges of most of the iodine-125 electrons are shorter than the length of the

adjacent follicular cell (5 um), as a consequence the electronic dose ($\mathscr{E}$) is highest at the follicular cell apex and lowest in the interfollicular stroma. The fall off in electronic dose across the cell is substantial and the total dose to the basal end of the cell and interfollicular tissue is low compared with that at the cell apex. The doses to the follicular cell nuclei are about 50 per cent of those to the cell apices and 50 per cent of the "mean" gland dose.

The pattern of inhomogeneity of dose distribution from iodine-125 shown in Fig. B-6 is potentially of considerable radiobiological importance, since the parts of the follicular cell complex presumed to control replication (nucleus) receives about half the dose to the parts responsible for the final stages of hormonogenesis (cell apex and microvillous margin). In addition, because about 85 per cent of the radiation dosage from any given colloid core is electronic and is confined to the parent follicle, mutual irradiation between follicles arises only from X-irradiation. It follows that if there is irregular iodine-125 accumulation which could arise because of inequality of follicular function, this will result in unequal rad doses to the complementary follicular cell spheres a situation which is not a feature of iodine-131 irradiation.

Thus in biochemical/replicative terms it would appear that external X and iodine-131 irradiations are equal to parts of the cell making storing and resorbing hormone and responsible for

reproductive integrity; iodine-125, in contrast, appears to irradiate the cell parts involved in some aspects of hormonogenesis more than the presumptive site of cell reproductive integrity (nucleus).

These conclusions led me to investigate and compare the effects of the three irradiations first on hormonogenesis on the thyroid in vivo then on cell proliferation. In Section C the effects of X-irradiations, iodine-131 and iodine-125 irradiations on hormone formation, storage and resorption (mainly apical cell function) are described. In Section D studies are described the objectives of which were to look at several indices of cell proliferation before and after irradiation in normal rat thyroid and in that growing under the influence of a goitrogenic challenge so as to select indices which were appropriate to measurement of the specific effects of irradiation on cell proliferation or survival. Only external X-irradiation was used in the studies of Section D because of the precise control of dosage rate, total dose, homogeneity of dose and quality (R.B.E. = 1) In Section E the effects of external X-irradiation on thyroid cell proliferation were re-measured and compared with the effects of iodine-131 and iodine-125 irradiations. The data are examined in the light of the dosimetries described above.

In these studies (Sections C and E) emphasis was placed on evaluating whether the inhomogeneous radiation from iodine-125

across the follicular cells produced any greater effects on hormonogenesis relative to those on cell proliferation.

Dose-Rate and Quality.

At this point in the thesis the important radiobiological factors of dose-rate, and quality (predicted R.B.E.) of irradiation from iodine-131 and iodine-125 irradiations will not be discussed since this is best done with the experimental data to hand. However whereas the dose-rate of X-irradiation is rapid and steady at all doses, that from iodine-131 and iodine-125 is relatively slow but falls exponentially during delivery; the rate of fall being dependent on thyroid B.H.L. With the radioactive iodine, however, mean dose rates must be proportional to total doses and the dose-rates from iodine-131 (H.L. = 8.0 days) are higher than those from iodine-125 (H.L. = 60 days).

SECTION C

COMPARATIVE EFFECTS OF EXTERNAL X-IRRADIATION, IODINE-131 AND IODINE-125 IRRADIATIONS ON RAT THYROID HORMONOGENESIS IN VIVO.

INTRODUCTION.

In Section A the structure and hormonogenetic functions of rat thyroid were described (Fig. A-4). Thyroglobulin is synthesised within the follicular cells and a series of polypeptides are formed in this process. Non-iodinated thyroglobulin is transferred across the apical end of the cell and at the colloid-apical membrane interface iodination of tyrosyls in the thyroglobulin molecule takes place and in the colloid thyroglobulin M.I.T., D.I.T., T3 and T4 are formed.

Normal rat colloid thyroglobulin is a 19S protein when measured by sucrose density ultracentrifugation; colloid 19S thyroglobulin is eventually taken back into the apical end of the cell by the pinocytotic activity of the apical membrane microvilli. Within the cell resorbed 19S thyroglobulin undergoes lyzosome fragmentation and proteolysis with the release of free M.I.T., D.I.T., T3 and T4; M.I.T. and D.I.T. are deiodinated by dehalogenase enzyme and most of the iodine is re-used for the iodination of new thyroglobulin but the free T3 and T4 cross the follicular cell basal membrane into the circulation. It appears that all follicles, irrespective of dimensions, and all follicular cells are active in hormone formation, storage and resorption. As far as can be determined there do not appear to be populations of synthesising cells and resorbing cells.

In Section B it was shown that external X-irradiation and iodine-131 irradiations were homogeneously distributed throughout the rat thyroid, microscopically and ultramicroscopically, such

that both irradiations result in equal doses to the bodies of the follicular cells (Hormonogenesis) and to their nuclei (cell proliferation); in contrast iodine-125 irradiations deliver more radiation to the apical ends of the follicular cells than to their nuclei (Fig. B-6). These considerations (Figs. A-4 and B-6) led logically to a comparison of the effects of each types of irradiation on different aspects of hormonogenesis in rat thyroid in vivo and the appropriate studies are now described.

The studies to be described repeat, complement or add to previous investigations of the effects of X-irradiation and of iodine-131 irradiation on rat thyroid hormonogenesis in vivo (Doniach and Logothetopoulos 1955, Abbatt, Doniach, Howard-Flanders and Logothetopoulos 1957, Al-Hindawi and Wilson 1965, Crooks, Greig, Macgregor and McIntosh 1964, Maloof, Dobyns and Vickery 1952, Taurog, Evans, Potter and Chaikoff 1960, Vittorio and Allen 1960, Anbar and Inbar 1963, Jovanovic, Djurdjevic and Sinadinovic 1965) and in vitro (Barzelatto, Murray and Stanbury 1962, Hall and Grand 1962).

Most of the studies of the effects of iodine-125 on rat thyroid hormonogenesis are, however, original. Up till the end of 1969 only one preliminary report of the effects of iodine-125 on thyroid hormonogenesis was found; Gross, Ben-Porath, Rosin and Bloch (1968) suggested that iodine-125 had preferential effects on rat thyroid hormone secretion as judged by poor body growth and increased size of pituitary but without loss of thyroid follicular cell D.N.A. The results of the current investigation will be

discussed with reference to the studies of Gross et al. (1968).

Principles, Objectives and Difficulties.

Principles and Objectives.

Two definitive experiments (1 and 2) were carried out. In each experiment groups of normal adult rats had their thyroids irradiated in vivo with graded doses of external x-rays or iodine-131 or iodine-125 irradiations. Within each experiment there was a non-irradiated control group, and a dosimetric subexperiment to obtain data for calculation of iodine-131 and iodine-125 absorbed doses as described in Section B. This included calculation of mean thyroid dose and microscopic distribution of dose by assuming that the structure of the thyroid could be represented by the model depicted in Fig. B-6. After the three irradiations had been delivered (4 weeks after delivery of the external x-rays and 6 weeks after administration of the isotopes of iodine), all rats in definitive experiments 1 and 2 were started on an iodine-deficient diet and distilled water. This regime was continued for a total of 3 weeks so that at the end of this interval "old" colloidal thyroglobulin present before and during irradiation would be replaced by "new" colloidal thyroglobulin formed after the irradiation (Schneider 1964). The iodine deficiency regime also ensured that all aspects of hormonogenesis, thyroglobulin synthesis, thyroglobulin iodination and thyroglobulin resorption proteolysis and hormone release and secretion were accelerated in rate and more amenable to measurement.

A phase of iodine deficiency lasting 3 weeks is a compromise; this duration was chosen to facilitate the measurement of fresh hormonogenesis but to minimise possible qualitative changes in hormonogenesis not due to the preceding irradiation but due to excessive follicular cell stimulationby T.S.H. and to minimise new cell production.

Experiment 1 - Post-Radiation Thyroglobulin Synthesis.

At the end of the phase of iodine deficiency, rats in definitive experiment No.1 were used to measure relative rates of new thyroglobulin synthesis and to measure the amounts of thyroglobulin present in the glands. The relative rates of new thyroglobulin synthesis were measured using an in vivo pulse label of the radioactive protein amino-acid precursor tritiated-leucine (H3-L) with sampling of thyroid after 4 hours, an interval which allows incorporation of H3-L into 19S thyroglobulin. The glands were excised and examined for total H3-L incorporation (total gland H3-L) and for relative H3-L incorporation into 19S thyroglobulin by the profile H3-L counts in 30 - 40 sucrose density ultracentrifugation (u.c.) protein fractions of rat homogenate (Thomson and Goldberg 1968, Thomson and Bissett 1969). The amounts of chemical 19S thyroglobulin in the fractions were determined using optical density spectrophotometry, running sheep thyroglobulin as the standard for mature 19S thyroglobulin. The H3-L content of homogenate and each u.c. fraction was determined by liquid scintillation spectrometry. The profiles of absorbance and of H3-L radioactivity of each u.c. sample were graphed simultaneously

for comparison. The absorbance at 19S (sheep thyroglobulin) gave an index of the actual chemical amount of 19S thyroglobulin in the gland at the time of sacrifice and the total H3-L radioactivity uptake and pattern into 19S thyroglobulin gave an index of the degree and normality of the rate of 19S thyroglobulin synthesis at the time of H3-L administration.

Experiment 2 - Post-Radiation Thyroglobulin Synthesis, Iodination, Composition and Resorption.

In definitive experiment No.2 and at the end of the 3 week phase of iodine deficiency all the rats were continued on the iodine-deficient diet and distilled water but tracer amounts of iodine-125 and stable potassium iodide were added to the distilled water. This regime was continued for 5 weeks to produce equilibrium labelling of more than 90 per cent of the intrathyroidal iodine whether it was free iodide, bound in colloidal thyroglobulin as M.I.T., D.I.T., T.3 or T.4 or whether the latter were free (Rhodes and Buddemeyer 1966, Loevenstein and Wollman 1967).

In definitive experiment No.2 thyroglobulin synthesis was measured as 19S thyroglobulin in u.c. fractions as described in experiment No.1. In addition, however, indices of thyroglobulin iodination, thyroglobulin composition and thyroglobulin resorption and proteolysis were also obtained. This was done, in principle, as follows. At the end of the phase of equilibrium labelling the rats were sacrificed and the relative 19S thyroglobulin in the thyroids determined by absorbance measurements on u.c. protein fractions using 19S sheep thyroglobulin as the standard.

To obtain an index of iodination, the iodine-125 content, of each u.c. fraction was also determined; the absorbance measurements gave indices of 198 thyroglobulin synthesis and the apposite iodine-125 counts gave indices of iodination AT equilibrium labelling. The profiles of the absorbance pattern and the iodine-125 tracer counts were therefore graphed simultaneously for comparison.

To assess thyroglobulin composition, resorption and proteolysis additional samples were taken from the pooled gland homogenates and the following indices were obtained. Total iodine-125 radioactivity of the gland was calculated (from a sample of the homogenate). Radiochromatographic (iodine-125) proportions of iodide, M.I.T. plus D.I.T. (iodotyrosines), T3 and T4 (iodothyronines). The total iodine-125 radioactivity of the glands gave indices of thyroid iodine accumulation and retention after irradiation. Radiochromatographic analysis, by providing semi-quantitative data on the relative amounts of free iodide, iodotyrosines (M.I.T. and D.I.T.) and iodothyronines (T3 and T4) in the thyroid gave combined indices of the iodine composition of thyroglobulin, thyroglobulin resorption and proteolysis.

Additional Measurements in Experiments 1 and 2.

At each sacrifice and in both experiments No.1 and No.2 total thyroid weights and total thyroid proteins (chemical) were measured. These data gave crude indices of gross structural changes brought about by the preceding irradiation.

Also at each sacrifice and in both experiments No.1 and

No. 2 heart blood was taken from each rat and pooled (each radiation treatment group) for chemical estimation of serum protein bound iodine-127 concentration (P.B.I.). The serum P.B.I. was taken as an index of average thyroid hormone (T4 and T3) secretion after irradiation.

Difficulties Encountered in Preliminary Experiments.

The only difficulty, which remained unsolved, was the assay of H3-L following iodine-125 irradiations as planned for experiment No.1. It was found even in rats given the smallest therapeutic dose of iodine-125 and after an iodine deficiency regime for 3 weeks that traces of iodine-125 persisted in the gland, appearing in the gland homogenates and in the u.c. fractions. This persistent contamination with iodine-125 made it impossible to distinguish and to simultaneously assay the small amounts of H3 from the pulse label of H3-L in the same samples.

Failure to distinguish H3 from iodine-125 arises because H3 and iodine-125 have electronic emissions within the same energy spectrum (Parmentier and Ten Haaf 1969). In addition for one disintegration iodine-125 emits many more low energy electrons than H3. Thus differential counting using conventional multiple channel simultaneous counting (liquid scintillation spectrometry) was not feasible and this was proven by several subsidiary experiments in vitro. Differential counting using the differential decay rates of iodine-125 and H3 was also tried but could not be adopted in practice, because after the samples containing the iodine-125 and the

H3 were prepared and counted for Count No.1 the samples deteriorated in respect of clouding, mixing and quenching, by the time Count No.2 could be meaningfully carried out. The half-life of iodine-125 is 60 days so that at least a 20 - 30 day interval had to be allowed between Counts 1 and 2 respectively. The possibility of assaying the iodine-125 content of the samples separately using gamma counting was considered since H3 is not a gamma emitter. It was, however, not possible to predict what the electronic count rate from iodine-125 should be in the liquid scintillation system knowing the gamma count.

Thus, several approaches were made to assay H3-L in gland samples after iodine-125 irradiation but each were unsuccessful because of the persistence of trace amounts of iodine-125. As a consequence meaningful data using H3-L pulse labelling for measuring thyroglobulin synthesis rate could not be obtained after iodine-125 irradiation in experiment No.1. Data on H3-L incorporation after no irradiation, X-irradiation and iodine-131 irradiation were, however, obtained and all the other data sought in experiments No.1 and No.2 were also obtained without procedural or technical difficulty.

Details of Experiments.

Experiment 1.

Radiation Effects on Thyroglobulin Synthesis Using Tritiated Leucine (H3-L).

Materials and Methods.

A total of 50 young adult male Sprague-Dawley rats (initial

body weight 150 - 200 G.) were used; they were supplied by A.J. Tuck and Son Ltd., England. Five groups of 4 rats each were used to calculate the radiation dosimetry of iodine-131 and iodine-125 respectively and the remainder, 15 pairs of two rats each, were used for the definitive experiment.

The 5 sub groups of rats used to calculate dosimetry were each given 5 uci of iodine-131 intraperitoneally and one group was killed at each of the following time intervals - 24 hours, 2 days, 4 days and 7 days. At each sacrifice thyroid weights and their iodine-131 contents were determined. In the definitive experiment, pairs of rats were given, no irradiation, 100, 500 and 1,000 rads of external x-rays, 5, 10, 20, 40 and 80 uci of iodine-131 by injection and 10, 40, 80, 160, 320 and 640 uci of iodine-125 by injection respectively. On the basis of the measured data from the sub groups given 5 uci iodine-131 and the amounts of iodine-131 and iodine-125 administered rad dose calculations as described in Section B were made; thus the mean absorbed radiation doses in the gland and the relative rad doses to different parts of the tissue and cells were calculated.

Four weeks after X-irradiation or six weeks after injection of iodine-131 or iodine-125 each pair of rats, including the non-irradiated pair, had standard diet (diet 41 pellets, Rowett Institute, Aberdeen) and tap water replaced by low iodine diet (Nutritional Biochemicals Corporation, U.S.A.) and distilled water respectively. This was continued for 3 weeks. At the end of this interval each rat (except those given iodine-125) was given

100 uci of H3-L (L-leucine 4-5T, Radiochemical Centre, Amersham TRA170) by single intraperitoneal injection. The specific activity was 1 Ci/m/M All rats were killed by inhalation ether 4 hours after the administration of the H3-L but before death, 3 ml of heart blood was taken from each pair of rats and pooled for chemical measurement of serum P.B.I. using the method of Farrell and Richmond (1961). Immediately after death the thyroid was removed, weighed to the nearest 0.1 mg. (Torsion Precision Balance) and prepared for chemical protein measurement (Lowry, Rosebrugh, Farr and Randall 1951) for total H3-L assay and for sucrose density u.c. analysis, each u.c. fraction being simultaneously assayed for selective absorbance at 280 u (using 19S sheep thyroglobulin standard) and H3-L incorporation. The methods were as described by Thomson and Goldberg (1968) and as modified by Thomson and Bissett (1969) in this laboratory.

The methodology was as follows: the thyroid glands from each pair of radiation and no-radiation treated rats were carefully cleared by hand of extraneous fibro-fatty tissue and finely minced on frozen clean glass plate. The minced thyroid was then homogenised (Tri-R Instruments model S63) in 1 ml of phosphate buffered saline (P.B.S. - 0.15 M sodium chloride in 0.01 potassium phosphate pH = 6.8). 0.2 ml of the homogenate was taken for chemical protein assay (Lowry et. al. 1951) and 0.1 ml was taken for total H3-L radioactive assay. For the latter 1 ml of 1 molar solution of hyamine hydroxide in methanol (Nuclear Enterprises Ltd.) was added to the homogenate and the digestant was assayed in a B.D.H. Toluene - 2,5-Diphenyloxazole (PPO)-1,4-Bis-

2-4 Phenyloxazolyl Benzene (POPOP) (Packard Instrument Co.). As described by White (1968) the H3 counts were made on a Packard Tri-Carb Liquid Scintillation Spectrometer (model 3375) with automatic correction for background and print out (IBM Electric). The final radioactive assay results were expressed in D.P.M. using the channels ratio method for correcting for inefficient counting.

The remainder of the homogenate (0.7 ml) was prepared for sucrose density u.c. analysis as described by Thomson and Goldberg (1968) and by Thomson and Bissett (1969). The homogenate was centrifuged at 15,000 r.p.m. for 10 minutes to remove cell debris. (Refrigerated Centrifuge Mistral 2L M.S.E.). The supernatant was not, however, made 50 per cent with respect to ammonium sulphate as described by Thomson and Bissett (1969) because attention was given only to 19S proteins. 0.2 ml of the homogenate and a sample of sheep 19S thyroglobulin was then applied to a linear 5-20 per cent sucrose in P.B.S. gradient. Each preparation was then ultra-centrifuged (Beckman Model Ultra-centrifuge L2-65B Class G) using an SW 39 Rotor, at 24,000 r.p.m. for 16 hours. The relative absorbance of each u.c. fraction was then measured at 280 u by passage through a Gilford 2000 Automatic Absorbance Recorder and 30 - 40 fractions (10 drops each) collected. The H3-L content of each fraction was measured in Brays Liquid Scintillant (Bray 1960) using the Tricarb. Liquid Scintillation Spectrometer with correction for background and efficiency as described above. The

sample of sheep 19S thyroglobulin spun in each rotor always gave discrete peak absorbance at 280 u. The point of highest discrete absorbance by the sheep thyroglobulin standard was therefore taken as the 19S thyroglobulin reference. The profiles of absorbance at 280 u and H3-L assays were graphed simultaneously for comparison. H3-L assays were not conducted on the u.c. fractions from iodine-125 irradiated rats for the reasons given above.

Experiment 2.

Radiation Effects on Thyroglobulin Synthesis, Iodination, Composition, Resorption and Proteolysis Using Iodine-125 Tracer.

In this experiment a total of 62 young male adult Sprague-Dawley rats were used (initial body weight 130-230 G). Five sub groups of 4 rats each were all given 5 uci iodine-131 intraperitoneally and a group killed at 24 hours, 2 days, 4 days, 6 days and 8 days respectively. At each sacrifice gland weight and radioiodine content (percentage dose given) was measured. The remaining rats were divided into 14 groups of 3 rats each for the definitive experiment. Two groups were not irradiated and four groups were given 125, 250, 500 and 1000 rads of external x-rays respectively; another 4 groups were given 5, 10, 20 and 40 uci iodine-131 intraperitoneally and respectively and the remaining 4 groups were given 40, 80, 160 and 320 uci iodine-125 intraperitoneally and respectively. The data from the dosimetric sub groups and the administered doses allowed radiation dosimetries to be calculated. All 14 groups were maintained under standard laboratory conditions

and diet for 6 weeks and then given the iodine-deficient diet and distilled water for a further 3 weeks.

Equilibrium Labelling with Iodine-125.

After iodine deficiency for 3 weeks the rats were commenced on the regime for equilibrium labelling of intrathyroidal iodide and iodine. This consisted of continuing the iodine-free diet but giving in addition, as drinking fluid, distilled water containing iodine-125 and potassium iodide (BDH-Anlar Grade). 25 litres were prepared containing 500 uci iodine-125 and 3750 ug potassium iodide and a fresh 25 litre tank was made up after 3 weeks. The equilibrium labelling procedure was continued for a total of 5 weeks as recommended by Rhodes and Buddemeyer (1966). This regime is estimated to produce at least 90 per cent equilibrium labelling in normal rat thyroid.

At the end of the phase of equilibrium labelling all animals were killed with anaesthetic ether but just before death 3 ml of heart blood was taken and the samples from each irradiation treatment group were pooled for measurement of serum P.B.I. (Farrell and Richmond 1961). Immediately after death the thyroid glands were resected, weighed to the nearest 0.1 mg. and pooled according to the preceding radiation treatment regime.

The pooled thyroid was prepared and homogenised in 1 ml of phosphate bufferred saline (P.B.S.) as described for experiment No.1. The iodine-125 content of 0.1 ml of this homogenate was determined to calculate the iodine-125 content of the thyroid pool, and then it was

used to measure the chemical protein content and so that of the thyroid pool (Lowry et. al. 1951). The remainder of the homogenate was used for u.c. fractionation, absorbance of each fraction at 280 u and its iodine-125 content being determined. The homogenate was prepared for u.c. fraction studies as described for experiment No.1, 0.2 ml. of supernatant being used and iodine-125 counts on the fractions being determined by well gamma counting. The profiles of absorbance at 280 u and iodine-125 counts on the u.c. fractions were graphed together for comparison. In the experiment sheep thyroglobulin was included as the standard for 19S thyroglobulin.

The remainder of the homogenate was used for radio-chromatographic analysis. After preliminary centrifugation the homogenate was prepared for iodine-125 radiochromatograms. The samples were concentrated (to 0.2 ml) by Carbo Wax (Polyethylene Glycol Mol. weight = 6000 - Koch Light). To this 0.2 ml. concentrate was added 0.2 ml. of 0.04 molar Tris/HCL buffer (pH = 8.0) whose composition was 0.30 molar NaCl, 0.08 Tris, 0.0004 molar $MnSO_4$, 0.004 molar Tapazole and 0.5 per cent pronase (Koch Light). The resulting solution was incubated at 37°C. for 6 hours. 100 ul samples of the digests were then analysed using descending paper chromatograms suspended in Butanol Acetic Acid Solution (Butanol: Acetic Acid: Water ratios = 78: 5: 17) for 16 hours at room temperature. With each thyroid radiochromatogram, known radioiodide, radio-iodotyrosine (M.I.T. and D.I.T.) and radio-iodothyronines (T3 and T4) were run from markers. When the

paper radiochromatograms were obtained (Packard Radiochromatograms Scanner Model 7200 with recording ratemeter Model 385) the sections containing the origin, the iodide, the M.I.T. + D.I.T. and the T3 + T4 were cut out and their respective iodine-125 contents were determined. By addition, the total radiochromatogram radioactivities were determined and by calculation the proportions (per cent) of each of the discrete components were obtained. The results were expressed, using the percentage of total radiochromatogram radioactivity for definitive data; since each thyroid radiochromatogram was standardised to 100 per cent comparisons between the different radiation treatment groups could be made. The basis of these methods are given by Stanbury, Kassenaar, Meiter, and Terpstra (1955), McGirr and Hutchison (1953, 1955), McGirr, Hutchison and Clement (1956, 1959) and by Pitt-Rivers and Cavalieri (1964), and more recently they have been reviewed by Rhodes (1968) and Pitt-Rivers (1967).

Results and Interpretations.

Experiment 1.

Dosimetric.

The precise X-irradiation doses and the calculated iodine-131 and iodine-125 doses throughout and within the rat thyroid are shown in Table C-1. In the sub group used to get data for radioiodine dosimetric calculations the mean thyroid weight was 19.2 mg., the mean thyroid uptake of radioiodine was 16.1 per cent (of the administered dose) and the biological half-life (B.H.L.) of thyroid

TABLE C-1

Absorbed Radiation Doses in Rat Thyroid

-- Experiment 1.

Radiation/Amount	Rad Doses		
X-rays	Mean	Colloid inner - cell	Cell Nucleus
0	0	0	0
100	100	100	100
500	500	500	500
1000	1000	1000	1000
Iodine-131			
0 uci	0	0	0
5 "	1,490	1,490	1,490
10 "	2,980	2,980	2,980
20 "	5,010	5,010	5,010
40 "	10,020	10,020	10,020
80 "	20,040	20,040	20,040
Iodine-125			
0 uci	0	Approx 0	Approx. 0
10 "	730	" 730	" 365
40 "	2,910	" 2,910	" 1,455
80 "	5,060	" 5,060	" 2,530
160 "	10,120	" 10,120	" 5,060
320 "	20,240	" 20,240	" 10,120
640 "	40,480	" 40,480	" 20,240

iodine was 5 days. As described in Section B the calculations of mean absorbed doses (rads) to the rat thyroid take account of a surface loss of 20 per cent of the iodine-131 β-radiation but no correction for surface loss of iodine-125 electronic radiation is necessary; more than 95 per cent of the mean iodine-131 irradiation and about 85 per cent of the iodine-125 irradiation deposited in the rat thyroid is β or electronic irradiation; a 15 per cent shortening of thyroid B.H.L. was allowed for in the final calculation of dosimetries arising from the administration of the highest amounts of iodine-131 (20, 40 and 80 uci) and of iodine-125 (90, 160, 320 and 640 uci).

The mean rad doses given in Table C-1 refer to the average energy deposited within the rat thyroid and for external X-irradiation and iodine-131 irradiation these are also the doses to all parts of the gland and to every part of the follicular cells. For iodine-125 irradiation the mean doses in Table C-1 are also equivalent to the doses given to the region of the colloid follicular cell interface (see Fig. B-6, Section B). The doses from iodine-125 absorbed by the middle and outer third of the follicular cells, where the nuclei are situated, are however about 50 per cent of the mean doses and of those to the inner part of the cell. Likewise the doses to the interfollicular stroma and vascular network are lower than the mean doses to the gland from iodine-125.

Whereas the dose rates from external X-irradiation are high and steady and the same for all total doses, those from iodine-131

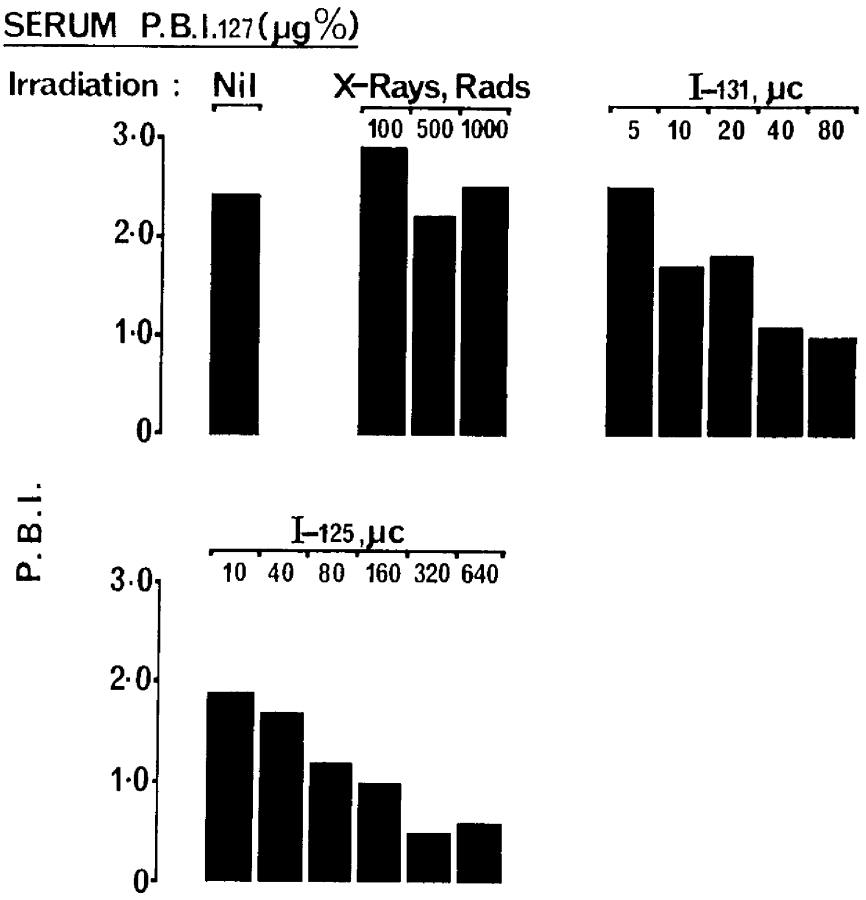


Fig. C-1. Chemical Serum P.B.I.127 per 100 ml. after irradiation of rat thyroid.

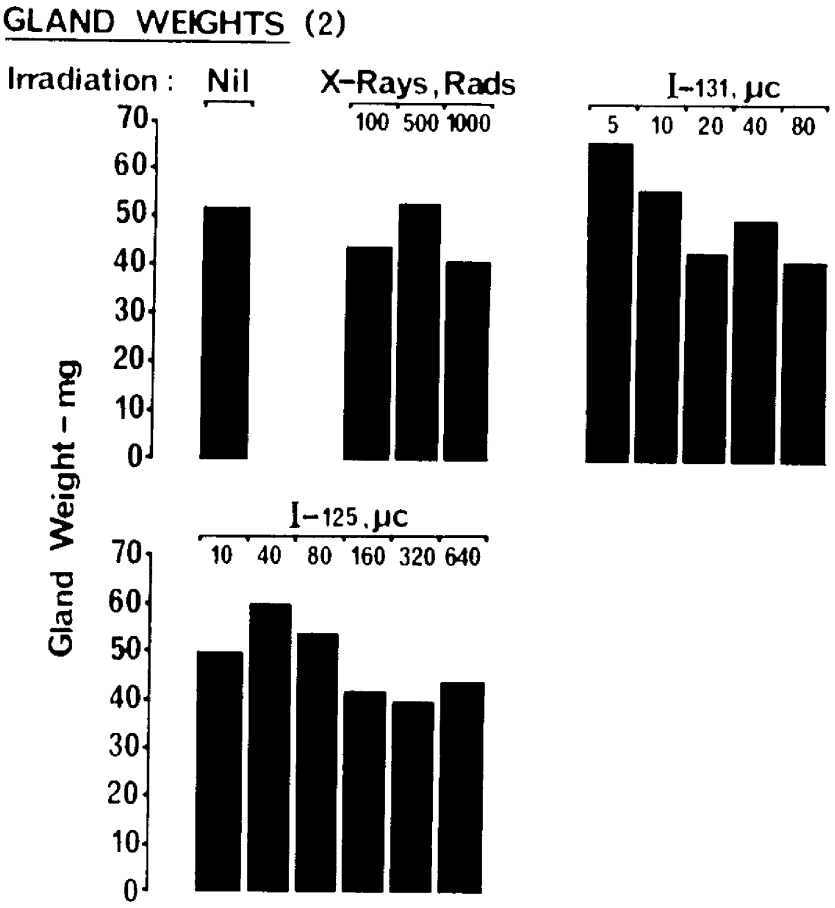


Fig. C-2 Rat thyroid weight (2 thyroids) after irradiation.

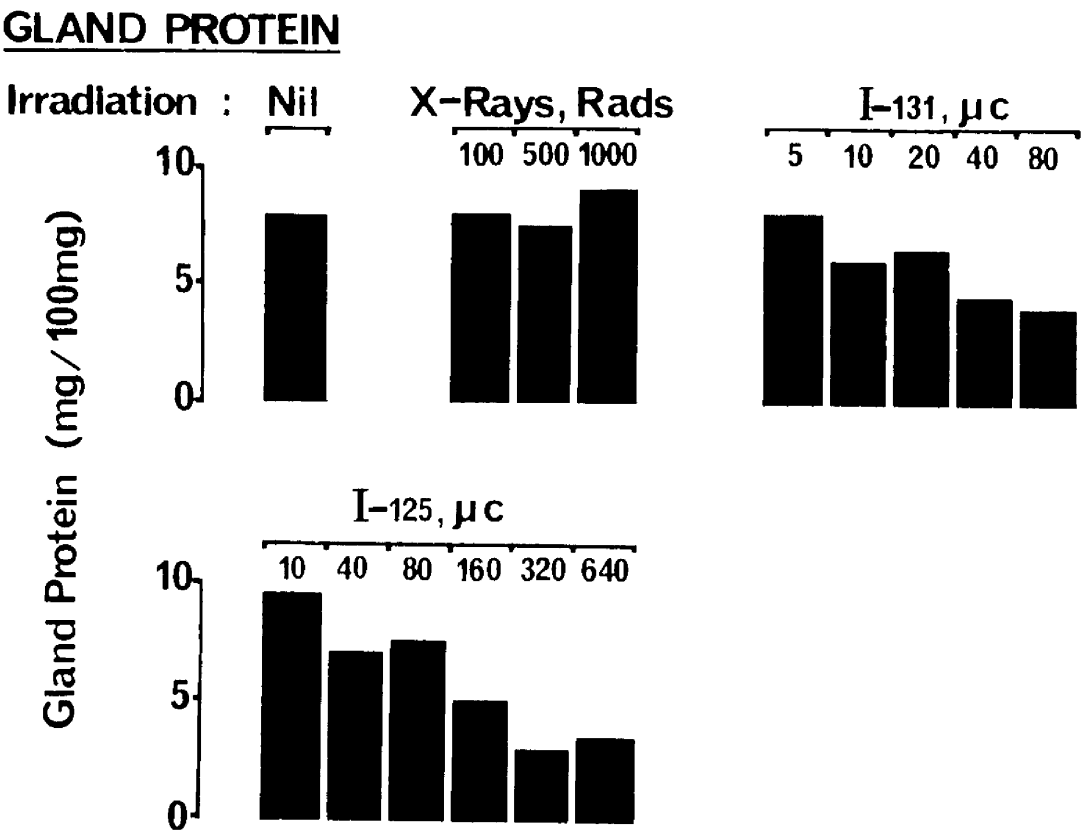


Fig. C-3 Rat thyroid protein (mg per 100 mg) after irradiation.

and iodine-125 fall exponentially but the "average" dose rates are proportional to total dosages.

Post-Radiation Measurements.

The data is given in Figs. C-1 to C-12 inclusive. In each Fig. the information is segregated according to the preceding irradiation regime; these were no irradiation, different doses of external X-irradiation and different amounts of administered iodine-131 or iodine-125. The absorbed radiation doses (rads) arising from the latter are of course given in Table C-1. In each Fig. the data refers to the mean of the two rat thyroids or the value obtained from pooled material.

Serum P.B.I.

Fig. C-1 shows the serum P.B.I. (ug per 100 ml) of sera pooled according to previous irradiation. None of the X-irradiation doses affected serum P.B.I. but both iodine-131 and iodine-125 irradiations lowered the serum P.B.I. the effect being greater as the doses of either isotope increased.

These data show, that X-irradiation in doses up to 1000 rads do not diminish net hormone secretion by rat thyroid, but iodine-131 and iodine-125 irradiations do and the effect is dose dependent. There is suggestive evidence that the highest doses of iodine-125 have a relatively greater effect on serum P.B.I. than doses of iodine-131 in the same range.

Thyroid Weight and Total Protein Content.

Fig. C-2 shows total gland weights (2 thyroids) and Fig.

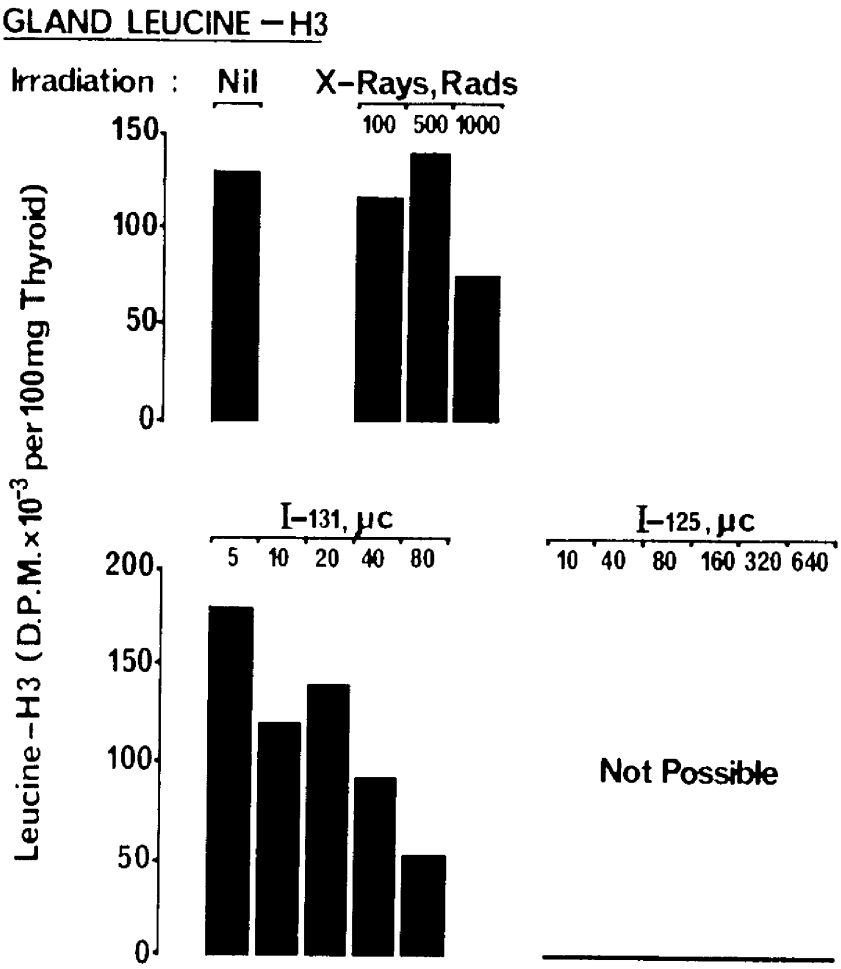


Fig. C-4 Incorporation of pulse label of leucine-H3 into rat thyroid after irradiation.

C-3 gland protein (mg per 100 mg thyroid). In both figures the data is segregated according to previous irradiation procedure.

Gland weight (Fig. C-2) was not affected by any of the irradiations and at all doses. Gland protein (Fig. C-3) was decreased only by the two highest doses of iodine-131 (40 and 80 uci respectively) and by the three highest doses of iodine-125 (160, 320 and 640 uci respectively.) The relevance of this will be discussed below.

Incorporation of Leucine-H3.

Fig. C-4 shows the leucine-H3 concentrations (D.P.M. per 100 mg of thyroid) and segregated according to the irradiations given. As indicated and discussed above, it was unfortunately not possible to measure leucine-H3 incorporation after iodine-125 irradiation.

As Fig. C-4 demonstrates the incorporation of leucine-H3 was diminished after 1000 rads of external X-irradiation and after 40 and 80 uci of iodine-131 but none of these doses abolished leucine-H3 incorporation. The effect, after the highest doses of iodine-131 was, however, dose related.

Profiles of Ultracentrifuge Protein Fractions - Absorbance at 280 u and Leucine-H3 Incorporation.

The profiles of absorbance at 280 u and Leucine-H3 incorporation were each measured on the same 30 - 40 u.c. fractions obtained from preparations of thyroid pooled according to preceding

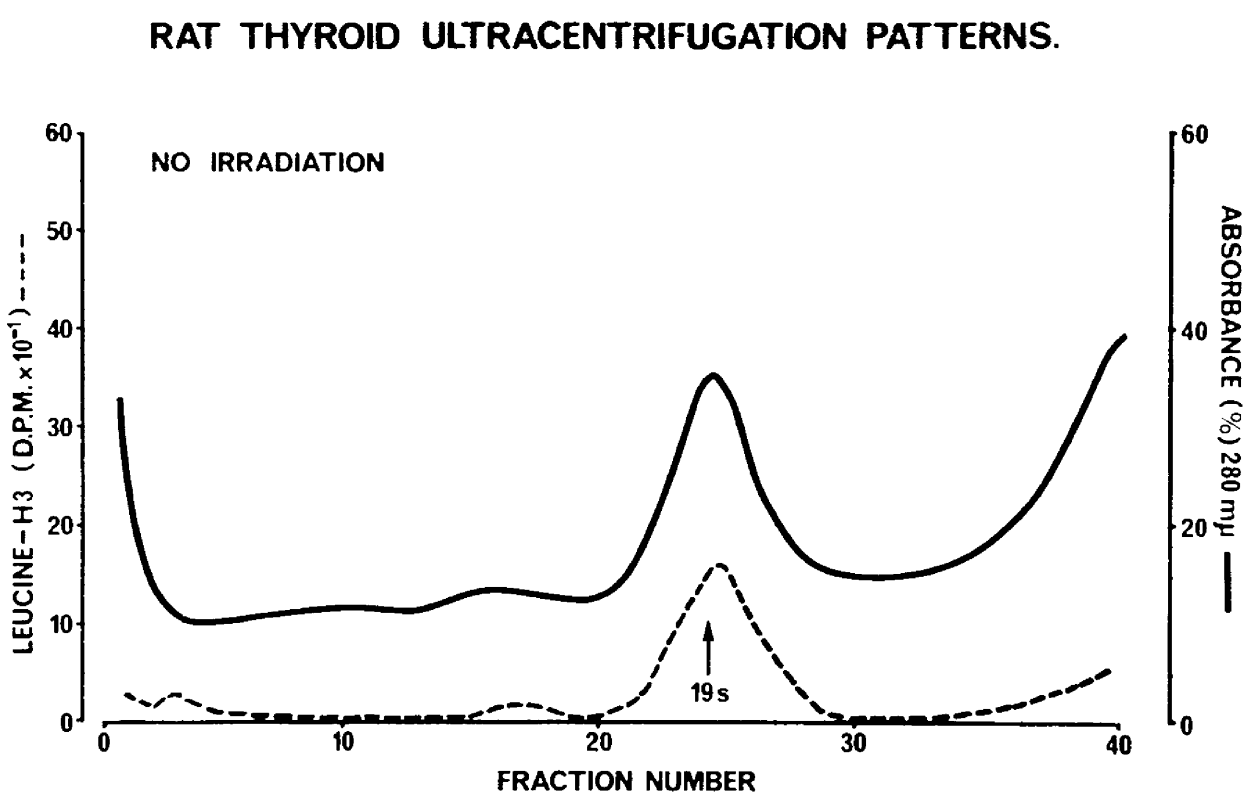


Fig. C-5 Per cent absorbance (280 mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - no irradiation.

RAT THYROID ULTRACENTRIFUGATION PATTERNS.

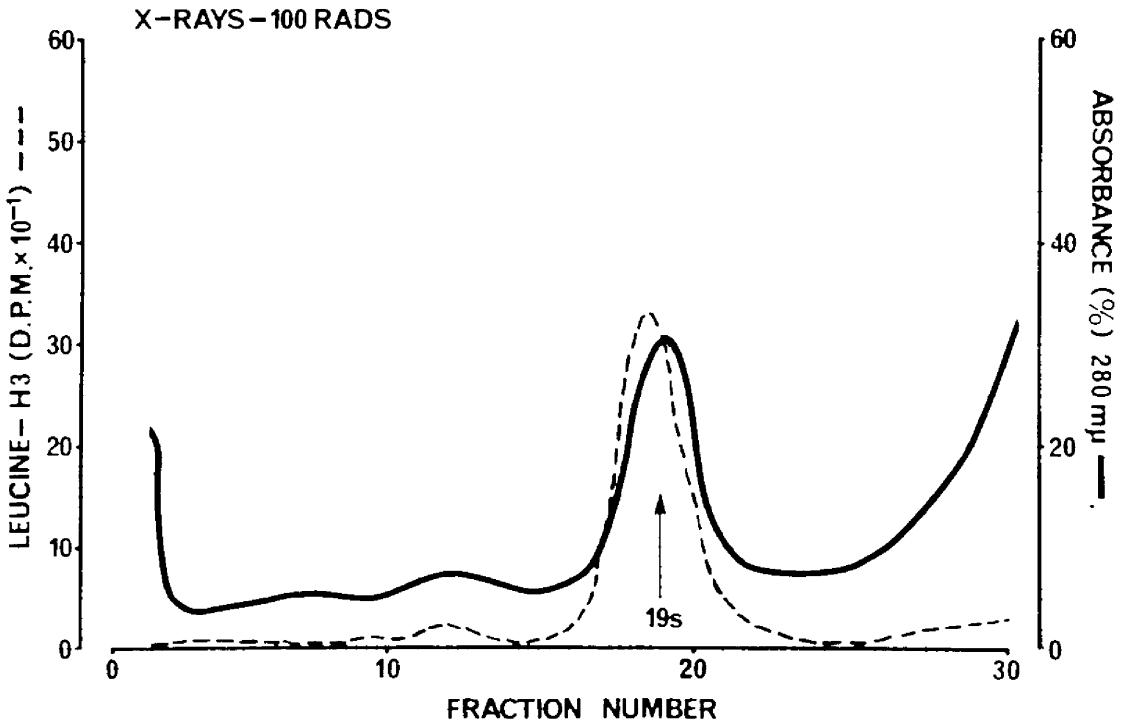


Fig. C-6 Per cent absorbance (280 mμ) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - after 100 rads x-rays.

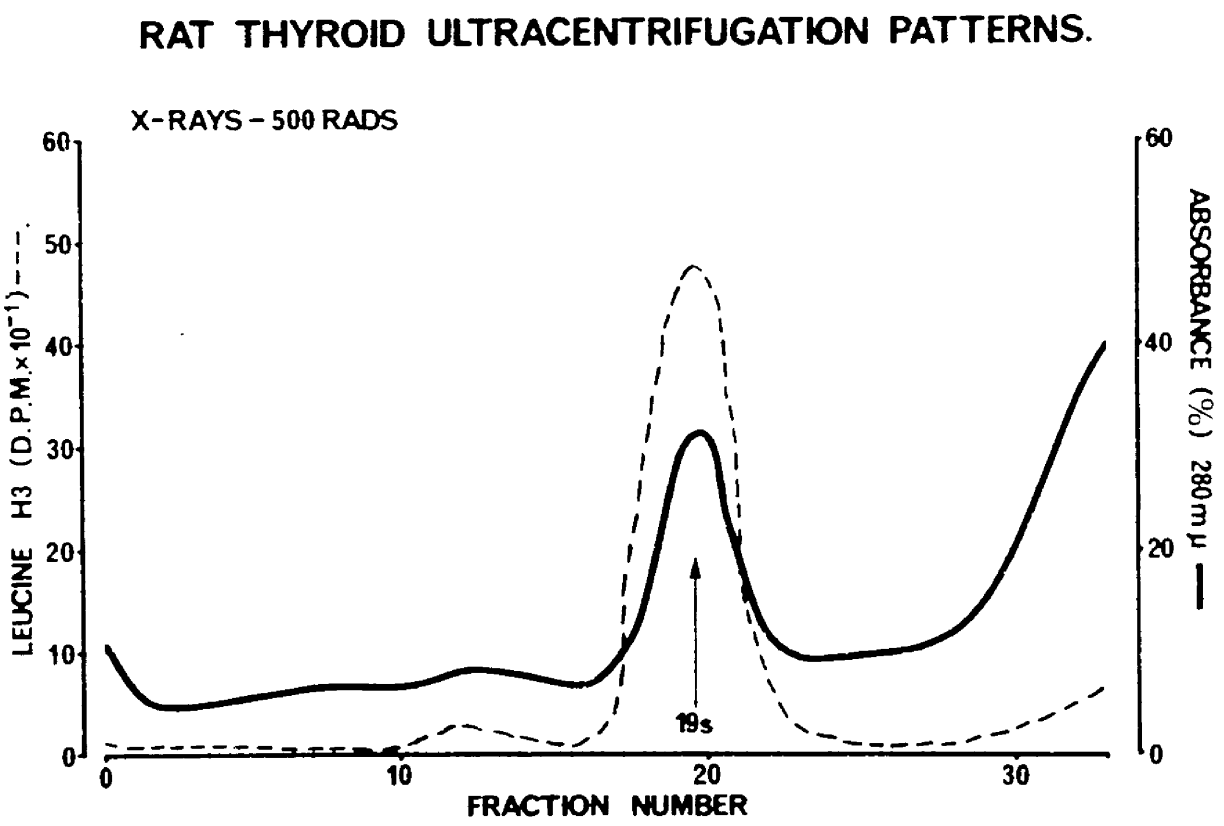


Fig. C-7 Per cent absorbance (280mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions – after 500 rads x-rays

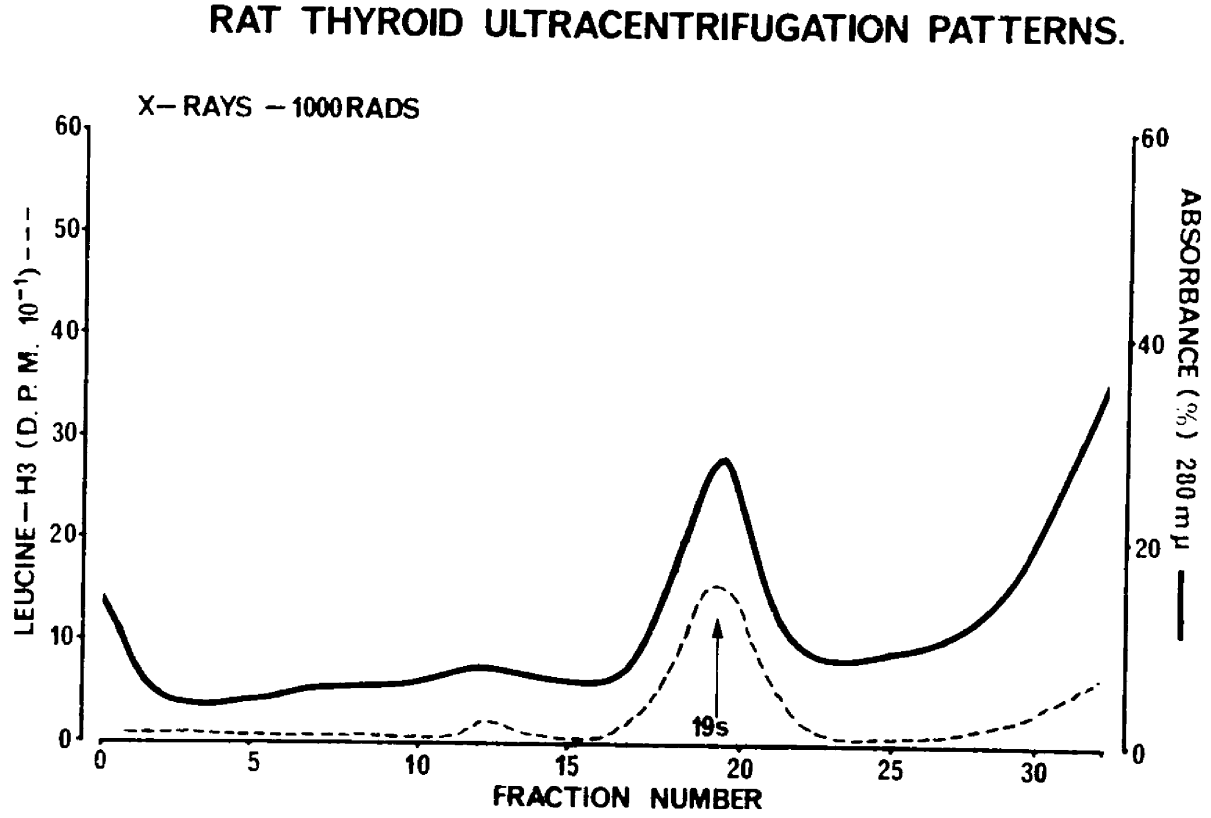


Fig. C-8 Per cent absorbance (280 mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - after 1000 rads x-rays.

RAT THYROID ULTRACENTRIFUGATION PATTERNS.

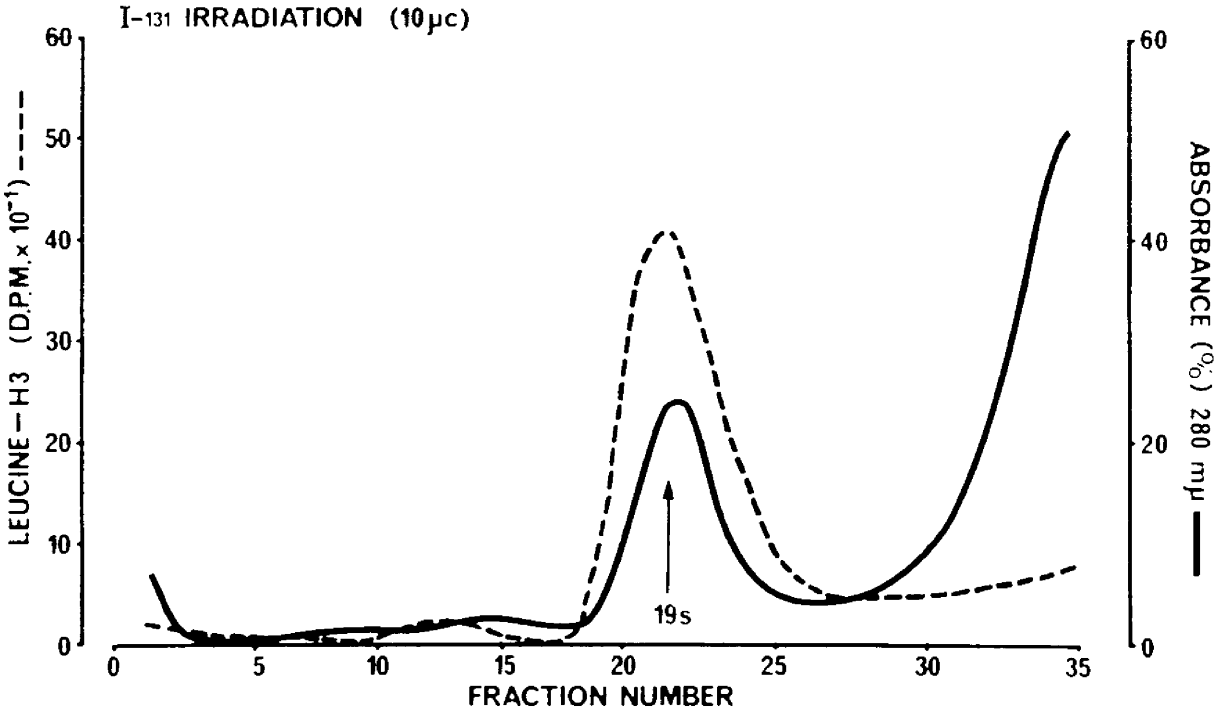


Fig. C-9 Per cent absorbance (280 mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - after 10 uci iodine-131.

RAT THYROID ULTRACENTRIFUGATION PATTERNS.

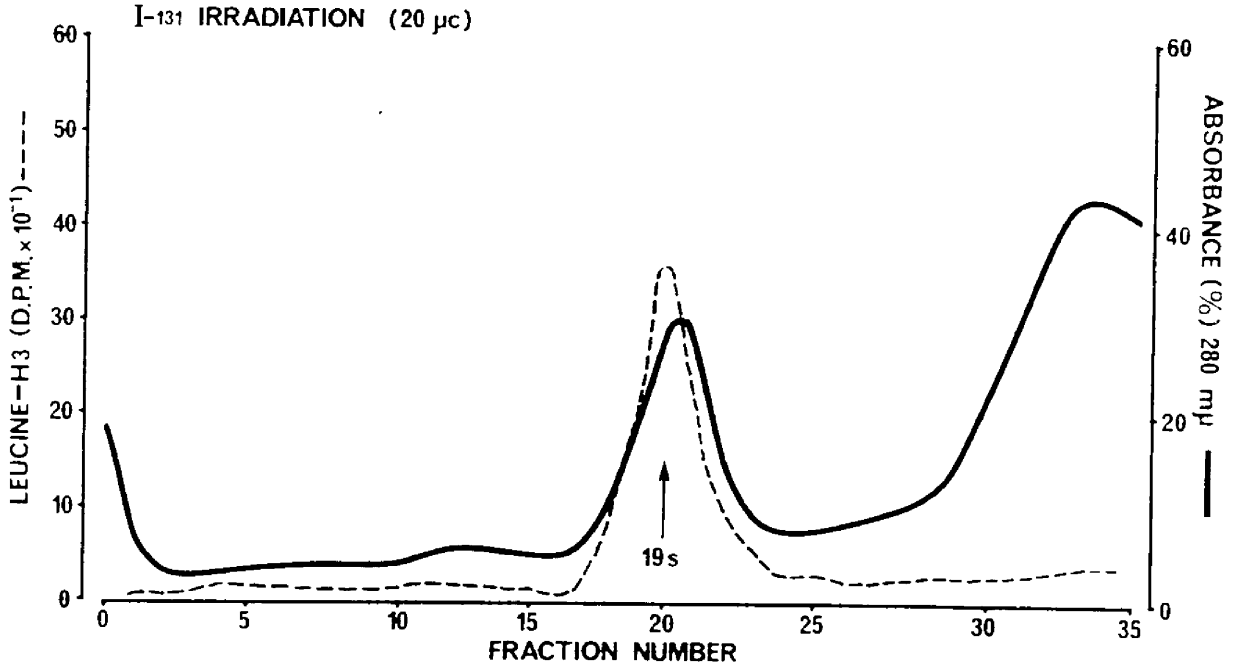


Fig. C-10 Per cent absorbance (280 mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - after 20 uci iodine-131.

RAT THYROID ULTRACENTRIFUGATION PATTERNS.

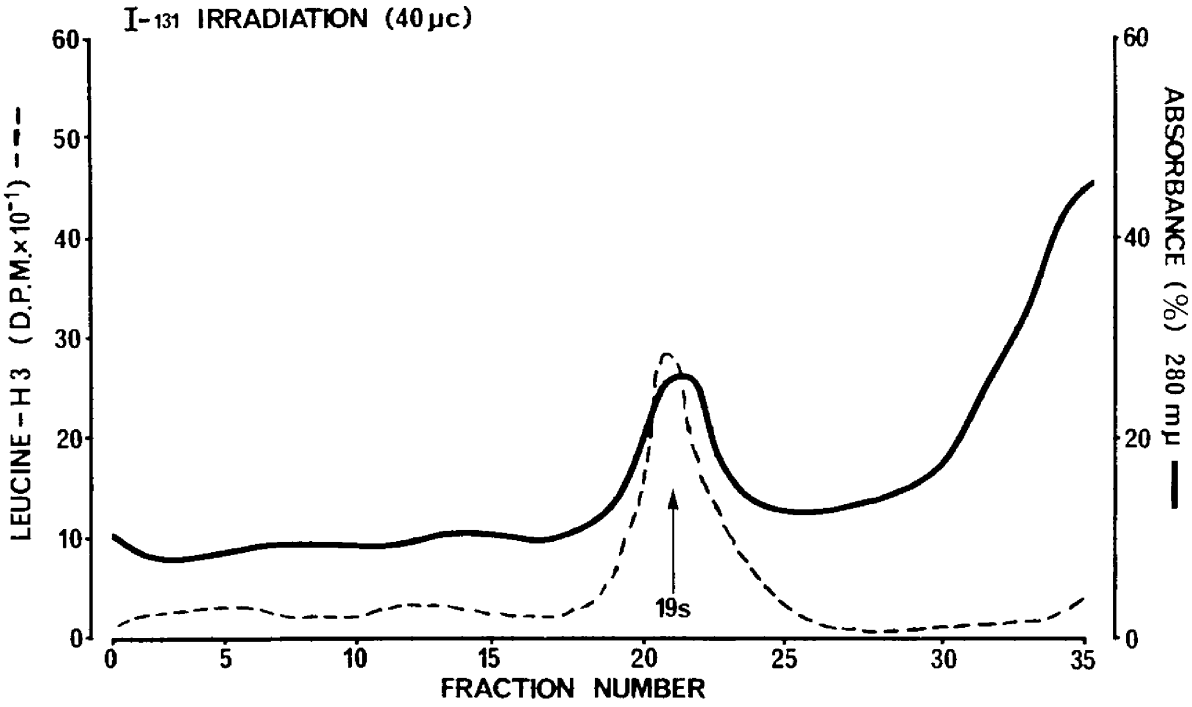


Fig. C-11 Per cent absorbance (280 mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - after 40 uci iodine-131.

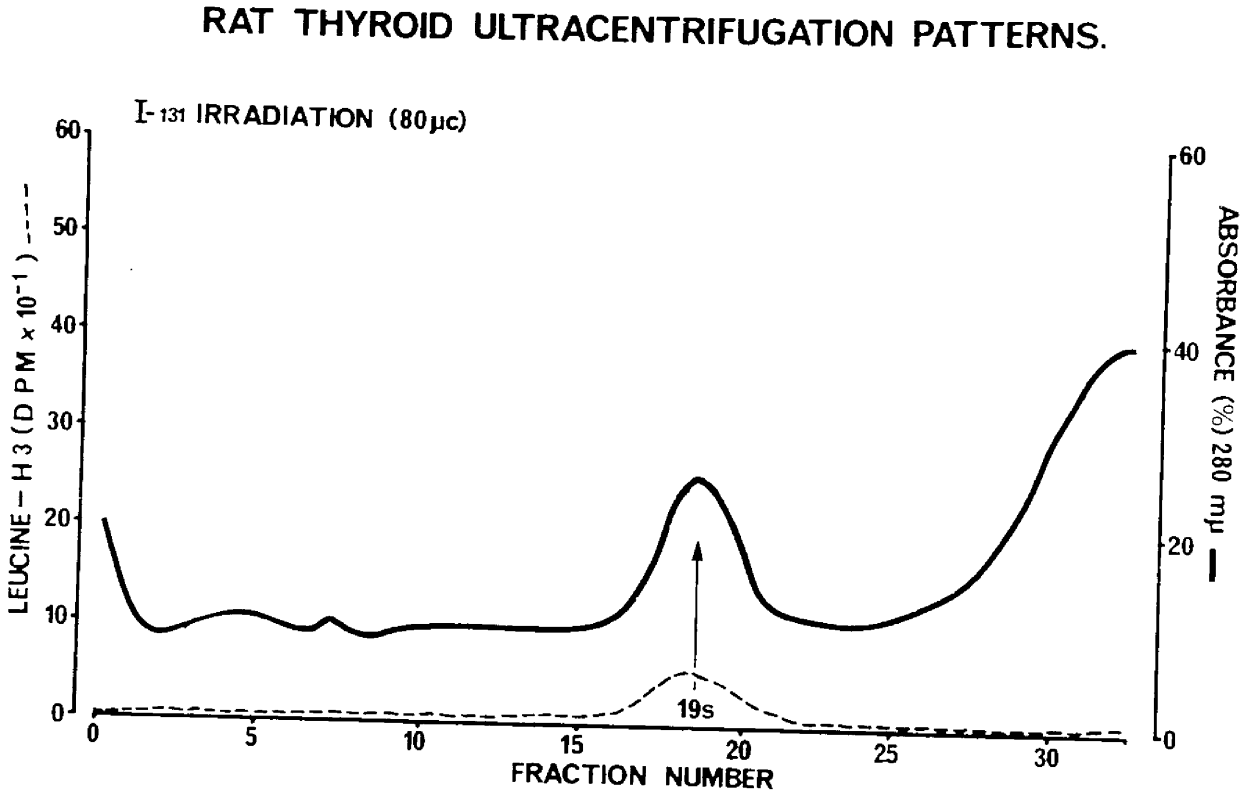


Fig. C-12 Per cent absorbance (280 mu) and leucine-H3 content (D.P.M.) of u.c. thyroid protein fractions - after 80 uci iodine-131.

irradiation. The absorbance profiles and the leucine-H3 profiles are therefore shown together in Figs. C-5 to C-12 respectively. Absorbance profiles are shown as continuous lines and the peaks obtained with the standard sheep 19S thyroglobulin, run at the same time, are shown as the 19S thyroglobulin references in each profile. The leucine-H3 content (D.P.M.) of the u.c. fractions are shown as interrupted lines. Profiles from rat thyroid not given irradiation (no irradiation) and from that given 100, 500 and 1000 rads of external X-irradiation and 10, 20, 40 and 80 uci of iodine-131 are shown in Figs. C-5 to C-12.

The data shown for u.c. protein fraction absorbance and leucine-H3 incorporation was obtained from pooled material and the methods used did not include salting out of blood proteins. Thus only semi-quantitative information is shown but special attention can be given to the 19S thyroglobulins. Thus unless gross changes are consistently demonstrated it is not possible to draw quantitative conclusions; nevertheless the data can be examined in a way meaningful to the question - does irradiation grossly alter the synthesis of 19S thyroglobulin (absorbance) or the rate of synthesis of thyroglobulin (leucine-H3 D.P.M.)?

There are differences in the heights and areas of the 19S peaks of absorbance, and leucine-H3 D.P.M. when the irradiated thyroid is compared to non-irradiated thyroid. In none of the irradiated thyroid, however, is there abolition of the discrete 19S absorbance at 280 u nor is there lack of a co-incident discrete content of leucine-H3. The main effects are after 1000 rads of

x-rays and 40 and 80 uc of iodine-131 (Figs C-8, C-11, C-12). Following these irradiations both 19S absorbance peaks and leucine-H3 D.P.M. peaks are low indicating that 19S thyroglobulin synthesis has been impaired.

As stated, although absorbance studies for 19S thyroglobulin were conducted after the iodine-125 irradiations, it was not possible to obtain the leucine-H3 content of the u.c. fractions because of insoluble counting difficulties. Since absorbance studies for 19S thyroglobulin after iodine-125 irradiations were repeated in experiment No.2 and the results were the same as those obtained in this experiment (No.1) the post-iodine-125 data is not shown here. The effect of iodine-125 irradiations on 19S thyroglobulin synthesis will, however, be shown and discussed in experiment No.2.

Preliminary Interpretation of Results of Experiment 1.

Experiment No.1 was designed first to measure the effects of external X-irradiation, iodine-131 and iodine-125 irradiations on net hormonogenesis (i.e. formation - storage - resorption and release of thyroid hormone) using serum P.B.I. as the index of net hormone secretion. The serum P.B.I. data in Fig. C-1 shows that in general X-irradiation up to 1000 rads do not depress hormone secretion but iodine-131 irradiation and iodine-125 irradiation can, the effects of the latter irradiations being seen after high doses, the depression in serum P.B.I. being dose related.

The weight and protein content data in Figs. C-2 and C-3 show that the gross structure (weight) of the thyroids was not

altered by any of the irradiations, and X-irradiation produced no alteration in protein content. The iodine-131 and iodine-125 irradiations, however, decreased the thyroid protein content, the effects increasing with dose. This crude measurement itself suggests that part of the explanation for the effects of the larger doses of iodine-131 and iodine-125 irradiations arise through decreased post-radiation synthesis of thyroglobulin. The data on rate of thyroglobulin synthesis measured by total uptake of H3-L into the thyroid shown in Fig. C-4 support this interpretation. Fig. C-4 shows a decrease in total leucine-H3 uptake only after the highest dose of external X-irradiation (1000 rads) but a progressively decreased uptake of leucine-H3 is observed after all doses of iodine-131 between 10 and 80 uci respectively.

Further evidence that thyroid irradiation decreases subsequent thyroglobulin formation and thyroglobulin formation rate is shown by the reduced 19S absorbance peaks and their leucine-H3 contents after 1000 rads x-rays and 40 and 80 uci of iodine-131.

It may be concluded, therefore, that whereas external X-irradiations in single doses up to 1000 rads do not impair normal rat thyroidal hormonogenesis as measured by serum P.B.I., they impair hormonogenesis as measured by thyroglobulin synthesis. In comparison the highest doses of iodine-131 irradiations reduce

TABLE C-2

ABSORBED RADIATION DOSES IN RAT THYROID

- EXPERIMENT 2.

Radiation/Amounts	Rad Doses		
	Mean	Colloid-Inner Cell	Cell Nucleus
X-rays			
0	0	0	0
125	125	125	125
250	250	250	250
500	500	500	500
1000	1000	1000	1000
Iodine-131			
0 uci	0	0	0
5 "	1550	1550	1550
10 "	3100	3100	3100
20 "	5210	5210	5210
40 "	10420	10420	10420
Iodine-125			
0	0	0	0
40	3030	Approx. 3030	Approx. 1515
80	5260	5260	2630
160	10530	10530	5265
320	21050	21,050	10525

serum P.B.I. and thyroglobulin synthesis. The highest doses of iodine-125 irradiations had as great an effect on serum P.B.I. as the highest doses of iodine-131 irradiations but since leucine-H3 studies were not possible after iodine-125 irradiations no conclusions can be drawn as to whether iodine-125 irradiations affected rate of thyroglobulin synthesis. The 19S thyroglobulin formation studies after iodine-125 irradiation not shown here but repeated and shown under experiment No.2, however, show that iodine-125 did not impair thyroglobulin formation (see below). The common and dissociated effects of iodine-131 and iodine-125 will be reconsidered in experiment No.2 in which serum P.B.I. 19S thyroglobulin synthesis were restudied after external x-rays, iodine-131 and iodine-125 irradiations. In addition data was obtained as to whether iodination of thyroglobulin was affected, and on thyroglobulin composition, resorption, proteolysis and release. This was obtained by radiochromatographic analysis for M.I.T., D.I.T. T3 and T4.

Experiment 2.

Dosimetric.

The precise X-irradiation doses and the calculated mean iodine-131 and iodine-125 doses throughout and within rat thyroid are shown in Table C-2. In the sub group used for data for these dosimetric calculations the mean thyroid weight was 21.2 mg., the mean thyroid uptake of radioiodine was 18.5 per cent and the mean B.H.L. of thyroid iodine was 5.2 days. The calculations take account of a surface loss of 20 per cent of iodine-131 irradiation

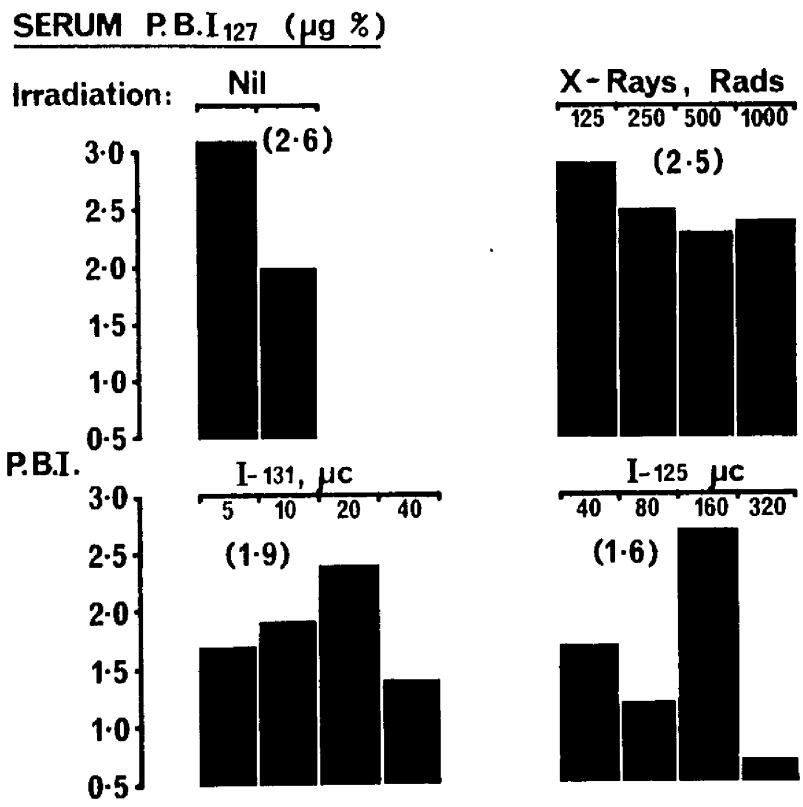


Fig. C-13 Chemical serum P.B.I.127 per 100 ml after irradiation of rat thyroid.

but no correction for surface loss of iodine-125 electrons was necessary. The dose calculations were, however, adjusted for a 15 per cent shortening of B.H.L. after the higher amounts of iodine-131 (20 and 40 uci) and iodine-125 (80, 160 and 320 uci).

As discussed in Section B the rad doses shown in Table C-2 and arising from X-irradiation and iodine-131 are the doses to all parts of the gland and to all parts of the follicular cells. The mean doses arising from iodine-125 also approximate those to the innermost parts of the follicular cells but the doses to the outer third of the follicular cells including the nuclei are about 50 per cent less (see Fig. B-6 - Section B).

Post-Radiation Measurements.

Fig. C-13 shows the P.B.I. of the sera pooled from three rats per radiation treatment group. The number in parenthesis is the mean of the P.B.I. for all radiation doses within each radiation category. The effects of the individual doses of irradiations on serum P.B.I. is variable and less definitive than was found in experiment No.1 (Fig. C-1); this variation was considered to be linked chiefly to the deliberate but artificial administration of potassium iodide to the drinking fluid for equilibrium labelling so that interpretation should not be based on individual values but on the mean P.B.I. for each radiation category. In this context Fig. C-13 shows that the mean P.B.I. of the two non-irradiated pooled sera was 2.6 ug per 100 ml. and the complementary values after X-irradiation, iodine-131 and iodine-125 irradiations were 2.5, 1.9 and 1.6 ug per 100 ml.

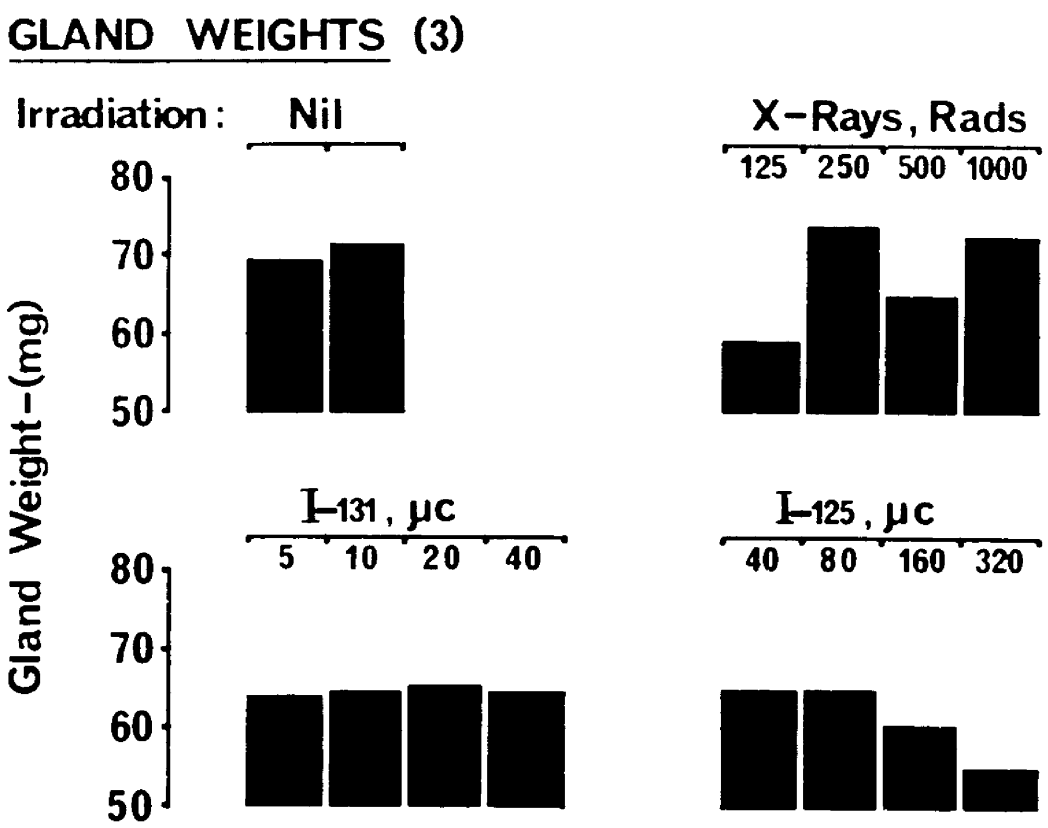


Fig. C-14A Rat thyroid weight (3 thyroids) after irradiation.

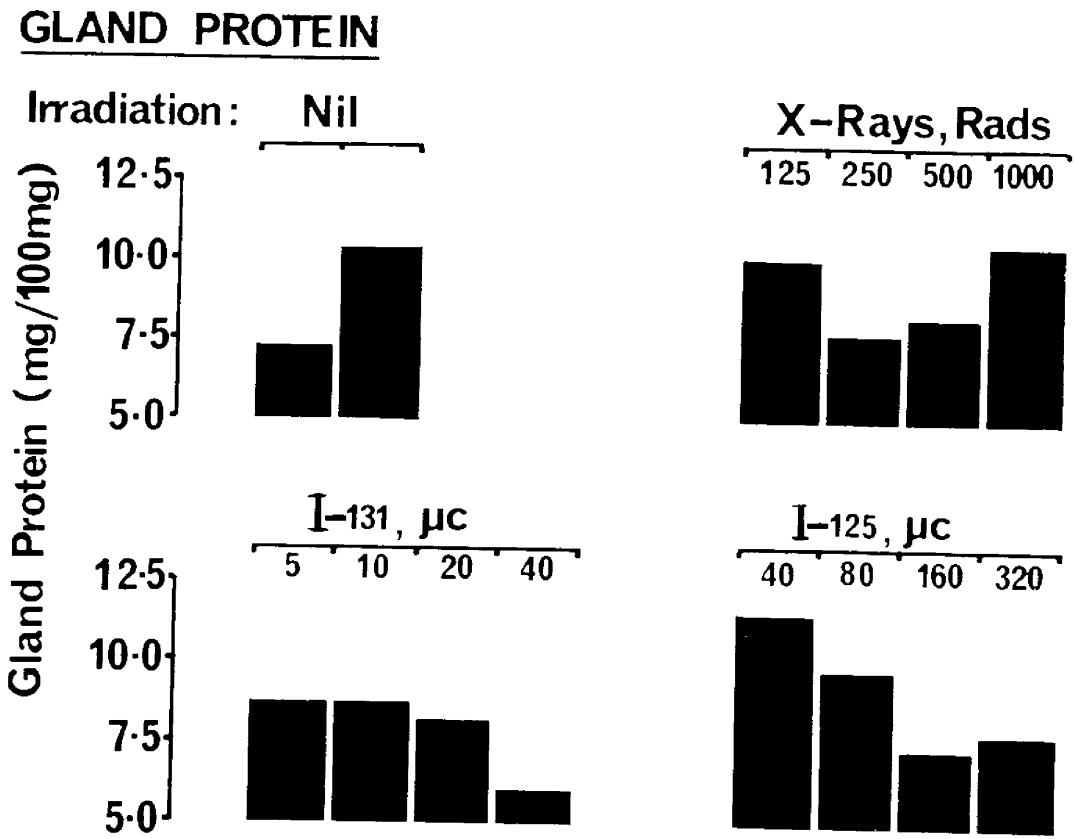


Fig. C-1[illegible] Rat thyroid protein (mg per 100 mg) after irradiation.

These data show that, in general X-irradiation did not affect hormone secretion while in general iodine-131 and iodine-125 irradiations resulted in a decrease in hormone secretion.

Thyroid Weight and Total Protein Content.

Fig. C-14A shows the aggregate thyroid weights (of 3 glands) per radiation treatment group. Although the aggregate weights have independent variation, the data does indicate that X-irradiation did not alter thyroid weight whereas iodine-131 and iodine-125 irradiations decreased it, the effect being greatest after the highest dose of iodine-125 (320 uci).

Fig. C-14B shows the protein content (mg per 100 mg thyroid) of the pooled 3 thyroids. Like the data on thyroid weight there is independent variation, but it may be concluded that X-irradiation did not affect total thyroid protein content whereas iodine-131 and iodine-125 irradiations caused a decrease, the effect increasing with dose.

Thyroid Iodine-125 Content.

Fig. C-15 shows the content of all iodine-125 (CPM per 100 mg thyroid) in the pooled thyroids (3) taken at the time of sacrifice. The radioactivity varied from 14×10^6 CPM to 40×10^6 CPM per 100 mg., the lowest activity being found in the thyroid irradiated with the highest dose of iodine-131 (40 uci) and the highest activity being found in the thyroid irradiated with the highest dose of iodine-125 (320 uci). In general, however, X-irradiation and iodine-131 irradiation did not alter the rat thyroids' subsequent concentration of tracer but after therapeutic iodine-125

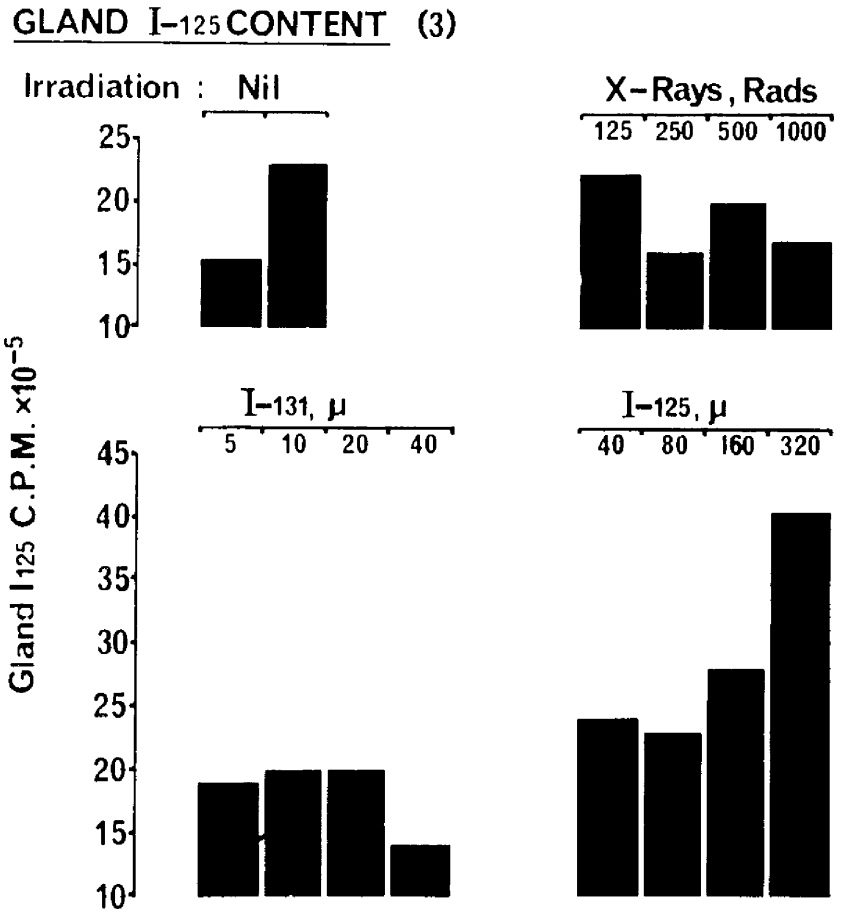


Fig. C-15 Iodine-125 content of pooled thyroid (3 thyroids) after a sequence of irradiation, iodine deficiency and equilibrium labelling.

irradiation, the amount of tracer iodine-125 found was high. This latter finding should be examined here.

It is possible, but unlikely, that part of the higher iodine-125 in the glands, previously irradiated with this same isotope, was residual. This statement is based as follows. The contribution from the therapeutic iodine-125 must, however, have been small because of the long interval between its administration and the sacrifice. This interval consisted of a 6 week lag between the injection and the start of iodine deficiency, a 3 week period of iodine deficiency and a 5 week phase of equilibrium labelling (total = 14 weeks).

After equilibrium labelling radioactive iodine = non-radioactive iodine. It is thus, provisionally, concluded that in general X-irradiation up to 1000 rads and in general iodine-131 irradiation did not decrease iodine content of the rat thyroid (except after 40 uci). In contrast after iodine-125 irradiation, the iodide content of the rat thyroid is increased the effect being dose related at high doses (160 and 320 uci).

Thyroglobulin Synthesis and Iodination - u.c. Studies.

The optical absorbances at 280mu (continuous lines) and the iodine-125 radioactivities (broken lines) of the same u.c. fractions are shown in Figs. C-16, C-17, C-18 and C-19. Since the material was taken from thyroids whose iodine was in at least 90 per cent equilibrium labelling with iodine-125 it can be assumed that the iodine-125 activities represent the actual non-radioactive iodine contents. The position of the 19S peaks

RAT THYROID ULTRACENTRIFUGATION PATTERNS.

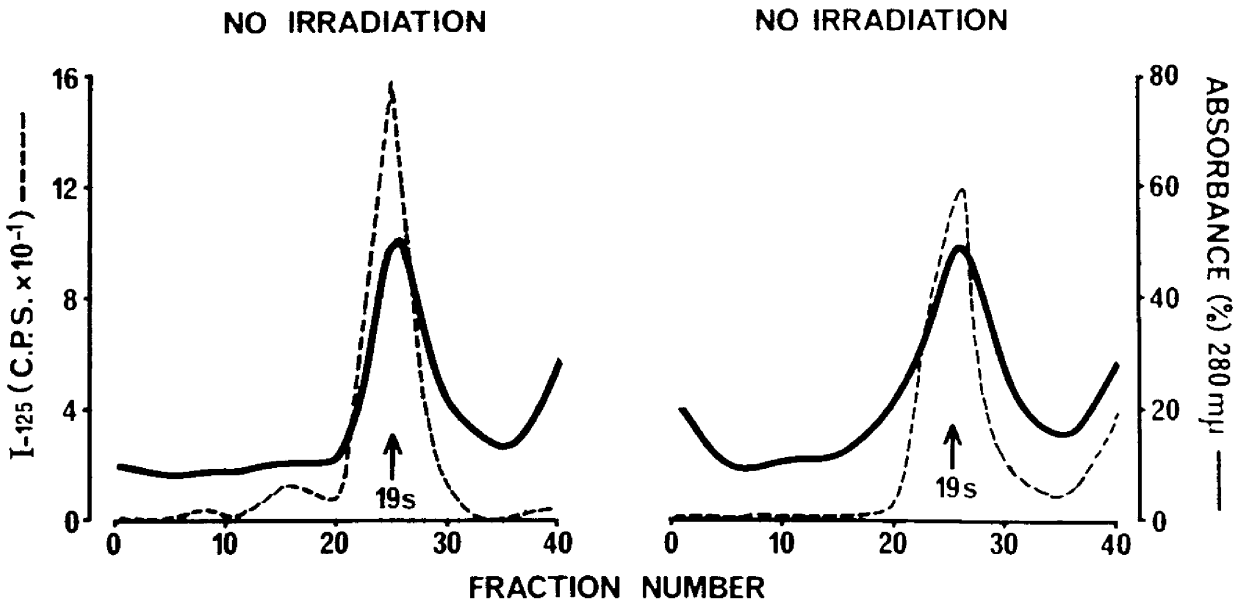


Fig. 6-16 Per cent absorbance (280 mμ) and iodination (I-125) of u.c. thyroid protein fractions - no irradiation.

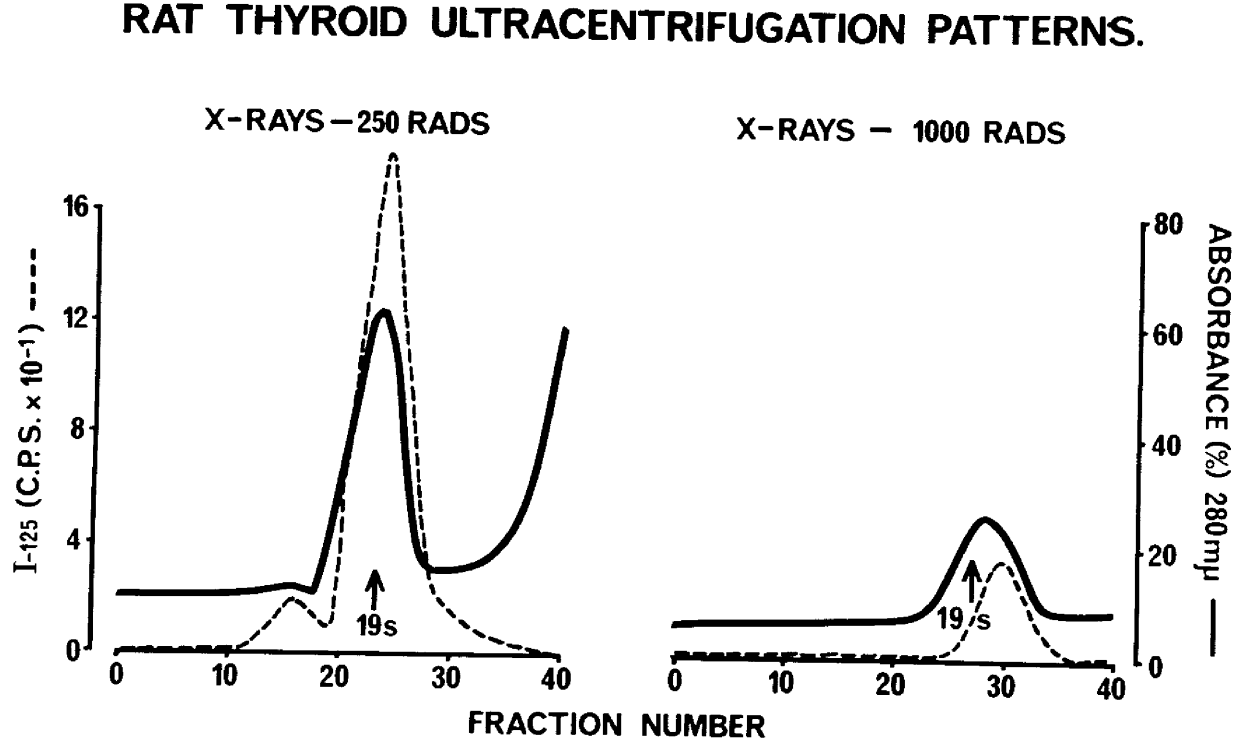


Fig. C-17 Per cent absorbance (280 mμ) and iodination (I-125) of u.c. thyroid protein fractions - after 250 and 1000 rads x-rays.

RAT THYROID ULTRACENTRIFUGATION PATTERNS.

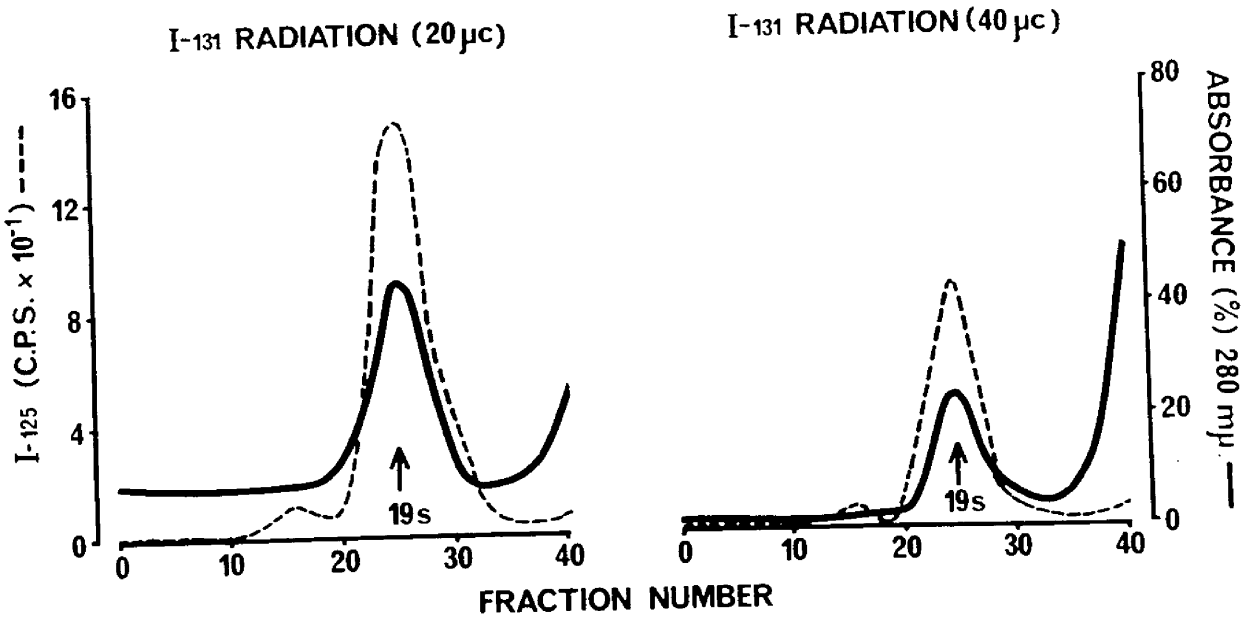


Fig. 6-18 Per cent absorbance (280 m) and ionization (I-125) of rat thyroid protein fractions - after 20 and 40 mc Iodine-131.

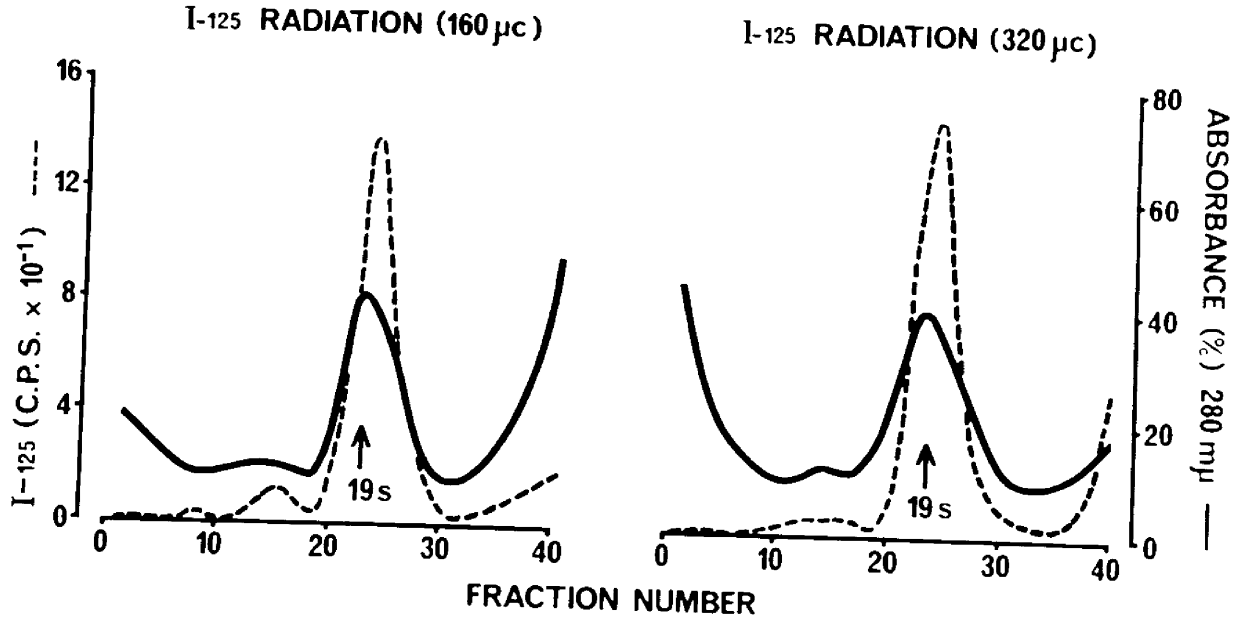


Fig. C-19 Per cent absorbance (280 mu) and iodination (I-125) of u.c. thyroid protein fractions - after 160 and 320 uci iodine-125.

obtained from the sheep 19S thyroglobulin are shown for reference. The 19S absorbances are rat thyroid 19S thyroglobulins and iodine-125 peaks co-incide. The profiles from the 2 non-irradiated groups (Fig. C-16) from the groups given 250 and 1000 rads of x-rays (Fig. C-17) from the groups given 20 uci and 40 uci iodine-131 (Fig. C-18) and from the groups given 160 and 320 uci iodine-125 (Fig. C-19) were chosen as representatives. In each non-irradiated and irradiated group the predominant pattern of absorbance and its iodine-125 radioactivity was 19S thyroglobulin. These are the measurements to be considered since they represent the amounts of mature thyroglobulin synthesised and its iodination.

Comparison of the X-irradiated (250 and 1000 rads) data (Fig. C-17) with the non-irradiated data shows (Fig. C-16), bearing in mind that the patterns are semi-quantitative, that 1000 rads reduced the amount of 19S thyroglobulin formed and its iodination. The patterns following 5 and 10 uci iodine-131 are not shown but were not different from non-irradiated thyroid (normal). The patterns following 20 and 40 uci iodine-131 are shown in Fig. C-18 and demonstrate that 20 uci iodine-131 had no effect but 40 uci did reduce the amount of 19S thyroglobulin formed and its iodination; even after 40 uci of iodine-131, however, the amount of iodinated 19S thyroglobulin detected was significant.

The patterns after iodine-125 irradiation (Fig. C-19) were, in contrast to those after 1000 rads x-rays and 40 uci iodine-131, normal at all irradiation dose levels. The patterns after 40 and

80 uci iodine-125 are not shown but those after 160 and 320 uci are in Fig. C-19. Comparison with the non-irradiated data (Fig. C-16). shows that after the highest doses of iodine-125, 19S thyroglobulin synthesis and iodination appear to be normal.

It may, therefore, be concluded that 19S thyroglobulin continued to be normally formed and iodinated after all doses of iodine-125, and even after the highest doses of x-rays and iodine-131 irradiation it was not abolished.

Thyroglobulin Composition Resorption and Proteolysis - Radiochromatograms.

One paper radiochromatogram was run from the preparation of pooled glands (3) from each non-irradiated and irradiated treatment group. The radioactive counts for the whole chromatogram and for each separate section, origin, iodide, M.I.T., D.I.T., T3 and T4 was determined. The data was standardised by expressing the radioactivity in each chromatogram section as a percentage of the total chromatogram radioactivity. Thus origin activity, iodide activity, iodotyrosine activity (M.I.T. + D.I.T.) and iodothyronine activity (T3 + T4) are expressed as percentages of total radiochromatogram activity per radiation treatment group. Since the data are from thyroids after at least 5 weeks equilibrium labelling the artefact of differential rates of labelling which might have arisen from a pulse label of radioactive iodine have been largely avoided. The data from the radiochromatograms (Fig. C-20 and C-21) are thus meaningful in terms of the proportion of stable iodide, stable iodotyrosines and stable iodothyronines in

RADIOCHROMATOGRAMS

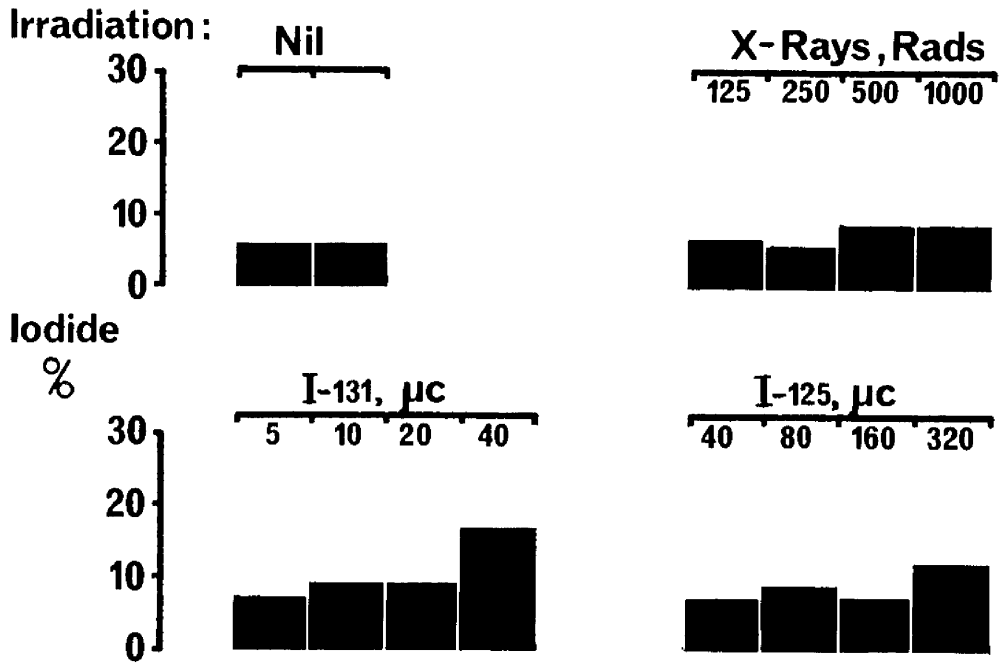


Fig. C-20 Per CENT iodide in Radiochromatograms of irradiated rat thyroid and after equilibrium labelling.

the thyroids and measured after irradiation.

Radiochromatograms - Iodide Phase.

Free iodide, as a percentage of total chromatogram activity for each irradiation dose is shown in Fig. C-20. The iodide phase varied from 6 to 17 per cent but there was no difference between the non-irradiated and the irradiated groups. The data confirm that about 10 per cent of the total rat thyroid iodine is iodide (see Section B and Halmi and Pitt-Rivers 1962) and irradiations from x-rays, iodine-131 and iodine-125 do not appear to modify this proportion.

Radiochromatograms - Iodotyrosines and Iodothyronines.

The percentages of the radiochromatogram activities due to iodotyrosines (M.I.T. + D.I.T.) and to iodothyronines (T3 + T4) are shown in Fig. C-21. The total height of the histograms are per cent iodotyrosines and the height of the hatched sections are per cent iodothyronines in the same chromatograms. The numbers below the histograms are the mean ratios of iodotyrosines to iodothyronines taking each radiation category together.

Iodotyrosines.

The label in the iodotyrosine phase (M.I.T. + D.I.T.) varied from 38 to 73 per cent. As Fig. C-21 shows the data from non-irradiated, X-irradiated and iodine-131 irradiated thyroid were similar. The iodotyrosine percentage after the highest dose of iodine-125 irradiation (320 uci) was, however, significantly lower than after all other irradiations and doses.

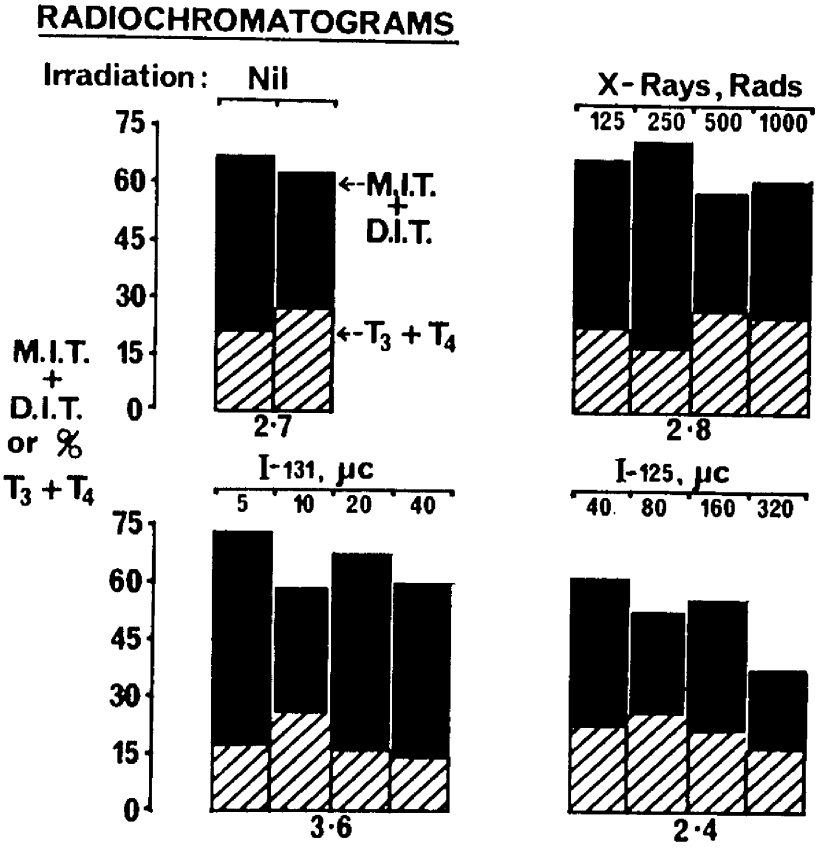


Fig. C-21 Per cent M.I.T. + D.I.T. and T3 + T4 in Radiochromatograms of irradiated rat thyroid and after equilibrium labelling.

Iodothyronines.

The label in the iodothyronine phase (T3 + T4) varied from 14 to 27 per cent. The data from non-irradiated and X-irradiated thyroid was similar. Iodine-131 irradiations, in general, decreased the percentage label in iodothyronines the effect being greatest at highest dose (40 uci). Iodine-125 irradiations, in general, however, did not decrease the percentage label in iodothyronines.

Ratio of Iodotyrosine to Iodothyronine.

The ratios given are means obtained from the discrete data from the two non-irradiated groups, from the four X-irradiated groups, from the four iodine-131 irradiated groups and from the four iodine-125 irradiated groups. The ratios given are average of the ratios within each radiation category. Only the iodine-131 irradiation group ratio (3.6) was different from that of the non-irradiated group ratio (2.7); the higher ratio after iodine-131 indicating a relative excess of iodotyrosines over iodothyronines. The group ratio after iodine-125 irradiations (2.4) was not different from the non-irradiated group ratio (2.7). The biological significance of these differences is uncertain however, and their detail will not be discussed except to state that the most striking feature of the data is the relative lack of gross effects of any of the irradiations or doses.

The iodotyrosine and iodothyronine labelling proportions in the rat thyroid after equilibrium labelling are equivalent to the actual proportions of iodine in these forms in the colloid thyro-

globulin and within the follicular cells. Thyroglobulin is either stored in the colloid or taken into the cell by the microphagocytic activity of the apical membrane where it undergoes lyzosome fragmentation and proteolysis with the release of its M.I.T. and D.I.T. and T3 and T4. The M.I.T. and D.I.T. are deiodinated by dehalogenase enzyme, the T3 and T4 are secreted into the blood stream at the basal end of the cell. The relative amounts of iodotyrosines and iodothyronines in the gland at a moment in time (sacrifice) are thus seen as an expression of the amount of M.I.T. and D.I.T. and T3 and T4 in colloid the thyroglobulin and the net balance between cell intake of colloidal thyroglobulin (apical membrane phagocytosis) the intracellular release of M.I.T., D.I.T., T3 and T4 (lyzosome fragmentation and proteolysis) and of deiodination of M.I.T. and D.I.T. and secretion of T3 and T4 respectively. The radiochromatographic data would appear therefore to indicate that the quality and quantity of these complex kinetic processes are not severely disrupted by X-irradiation, iodine-131 or iodine-125 irradiation. X-irradiation certainly has no effect but iodine-131 irradiation appears to reduce the amounts of T3 and T4 and iodine-125 irradiation reduces the amount of M.I.T. and D.I.T. It is not possible to take the interpretation of the data further except to state that the possible effects of iodine-131 and iodine-125 irradiations may be to either alter the iodotyrosine and iodothyronine composition of colloidal thyroglobulin itself or the phagocytic-proteolytic

action of the cell apex.

Whether irradiations have some specific or subtle effect on these processes discussed abovecannot, therefore, be resolved from radiochromatographic data. It appears, however, that in general the late stages of hormonogenesis namely storage proteolysis and hormone release within cells are not affected by X-irradiations up to 1000 rads and only partially impaired by iodine-131 irradiations (up to 40 uci) and by iodine-125 irradiations (up to 320 uci).

General Discussion on Effects of Irradiations on Hormonogenesis.

In Section A evidence was presented for the topographical location of structures responsible for mature thyroglobulin synthesis being at the inner third of the follicular cells and those for iodination at the innermost apical-colloid margin. In addition, in Section B, evidence was presented that X-irradiation and iodine-131 irradiation deliver homogeneous radiation doses to the hormonogenetic parts of the follicular cells and to the nuclei, to basal membrane and to interfollicular stroma; in contrast iodine-125 irradiations give the highest doses to the apices of the follicular cells, lower doses (about 50 per cent of mean) to the cell nuclei and lowest doses to basal membrane and interfollicular tissue.

These considerations, taken together with the magnitude of the absorbed rad doses shown in Tables C1 and C-2 suggest that the iodine-125 irradiations used might have selectively impaired mature thyroglobulin synthesis and/or its iodination. No such effects

were, however, found since in fact iodine-125 irradiation had less effect on thyroglobulin synthesis and iodination than X-irradiation or iodine-131 irradiation (Figs C-16 to C-19). This suggests that the current concepts of the location of these aspects of hormonogenesis might need to be revised. Perhaps the outer third of the follicular cells (containing the nucleus) and the basement membrane do play a significant role in dictating thyroglobulin synthesis and iodination and these structures, of course, are relatively spared after iodine-125 irradiation. Alternatively, the combination of a high radiation dose to the inner parts of the follicular cells and an equally high dose to the outer third (including the cell nucleus) and to the interfollicular stroma arising from X-irradiation and iodine-131 irradiation but not from iodine-125 irradiation might explain the relatively greater effects of x-rays and iodine-131. Nevertheless, irrespective of the differential effects of these irradiations on thyroglobulin synthesis and iodination it is a fact that iodine-125 irradiation did significantly impair hormone secretion as shown by the low rat serum P.B.I. (Figs. C-1 and C-13). A similar effect on serum P.B.I. followed iodine-131 irradiation in the higher dose ranges but external X-irradiations had relatively little effect. Thus whereas external x-rays and iodine-131 may impair 19S thyroglobulin synthesis and iodination and iodine-125 has little effect the differences are not necessarily reflected in hormone secretion. This tentatively suggests that iodine-125 may exert its chief influence not on hormone formation but on resorption of thyroglobulin

from colloid or thyroglobulin proteolysis and T3 and T4 release in and secretion from the cells. Lack of gross effects on M.I.T., D.I.T. and T3 and T4 proportions after iodine-125 irradiations does not exclude these possibilities.

The mode of action of iodine-125 irradiations in impairing rat thyroid hormone secretion may, therefore, lie outside on effect on mechanisms measured by the technicology used in the current study. It is quite feasible that the high and relatively selective irradiations given to the innermost parts of the follicular cells and particularly to their apical margin are critical. The localised electronic irradiations of iodine-125 may, for example, alter the biochemical and pinocytic functions of the apical membrane in a way which is not demonstrable in terms of the gross production of 19S thyroglobulin and its iodination or in terms of the crude proportions of iodotyrosines and iodothyronines but might be if more refined studies of thyroglobulin synthesis were conducted (Thomson and Bissett 1969) or additional selective measurement of, for example, apical membrane pinocytosis were included.

Comparison of Current Studies with Published Reports.

Many of the studies conducted in experiments No.1 and No.2 have not been reported previously and this applies especially to the effects of iodine-125. Some aspects of the studies have, however, been reported; for example, it has been recognised for many years that hormonogenesis *in vivo* in the rat thyroid is relatively radio-resistant (Abbatt et. al. 1957, Maloof et. al.

1952, Taurog et. al. 1960, Doniach and Logothetopoulos 1955) and more recently these conclusions have been confirmed (Jovanovic et. al. 1965, Crooks et. al. 1964, Al-Hindawi et. al. 1965). Studies in vitro also show that functions, related to general thyroid cell metabolism as well as to hormonogenesis are radio-resistant. For example, Barzellato et. al. (1962) found that external γ ray doses up to 500,000 rads failed to affect oxygen intake into thyroid slices; the same investigators showed that after 200,000 rads at least 50 per cent of iodine incorporation into proteins persisted and after 50,000 rads at least 50 per cent of leucine-H3 incorporation into proteins persisted. They also found that the ratio of M.I.T. to D.I.T. increased after irradiation of the thyroid slices. Hall and Grand (1962) using external γ-radiation and thyroid slices found that after 50,000 rads 50 per cent of formate incorporation into protein was retained. The same authors also showed, however, that incorporation of formate into purines was much more radiosensitive, a 50 per cent reduction being produced by 3500 rads γ rays. These studies clearly show that different aspects of cell function and hormonogenesis demonstrate different radiosensitivities.

The reported studies which most closely resemble the current studies are, however, the four papers by Jovanovic et. al. (1965) and by Djurdjevic, Jovanovic, Krainceanic and Sinadinovic (1969). They studied the effects of very large doses of iodine-131 (300 uci or more) on normal rat thyroid. They found that

iodide trapping and the conversion of iodide to iodine was very radio-resistant; they also found that after very large doses of iodine-131 irradiation, M.I.T./D.I.T. and T3/T4 formation continued but the proportion of the latter was decreased. Laborde, Commenay, Meyniel and Blanquet (1966) also found this effect after γ radiation. The current studies confirm this (Fig. C-20 and C-21). Djurdjevic et. al. (1969) studied the iodoproteins of the rat thyroid after very large doses of iodine-131 irradiations only. They found that the formation of 19S thyroglobulin was decreased but not abolished. They also studied the iodoproteins in the blood of rats after large doses of iodine-131 irradiation (300 uci) and found normal thyroglobulin and abnormal iodoproteins were released from the thyroid. These studies compliment those of Anbar and Inbar (1963) and Trianta phyllidis, Thomson, Barnaby and Jasani (1969) who found that irradiated rat thyroid appeared to produce a butanol insoluble iodoprotein or to iodinate (with reactive iodine) serum albumin as it passed through the gland, a phenomenon also noted by Stanbury and Janssen (1962) in the sera of thyrotoxic patients treated with irradiation. While the release of thyroglobulin after irradiation may be an accentuation of a normal leak (Torrigiani, Doniach, and Roitt 1969) the iodination of albumin appears to be abnormal.

The only report about the radiobiological effects of iodine-125 on rat thyroid, however, is a preliminary study by

Gross, Ben-Porath, Rosin and Bloch (1968). In general using simple parameters such as radioiodine uptake, pituitary weight and rat body growth they concluded that iodine-125 has a greater effect on hormonogenesis than on thyroid cell hyperplasia because the dose to the inner cell is greater than that to its nucleus. They postulated that iodine-125 probably exerts its effects chiefly on T3/T4 formation in the thyroglobulin. The current studies do not entirely disprove this but, as discussed above, make it unlikely to be the chief effect. My belief is that iodine-125 exerts its greatest effect on hormonogenesis by impairing thyroglobulin resorption. Thyroglobulin resorption requires regulated pinocytosis by the apical membrane and the latter is the point receiving the highest doses from iodine-125. Further direct studies are necessary, however, to prove this hypothesis.

In the next Section (D), rat thyroid cell proliferation and desoxyribonucleic acid (D.N.A.) synthesis are studied in normal rat thyroid and rat thyroid irradiated with external x-rays. The study was designed to obtain two groups of data; a group which might allow some insight into the cell mechanisms and structures involved when irradiation impairs cell proliferation (? nucleus) and a group which might allow selection of a simple parameter of radiation effects on thyroid cell survival (longevity and reproductive integrity). External X-irradiation was used since it is precisely controlled in respect of dose,

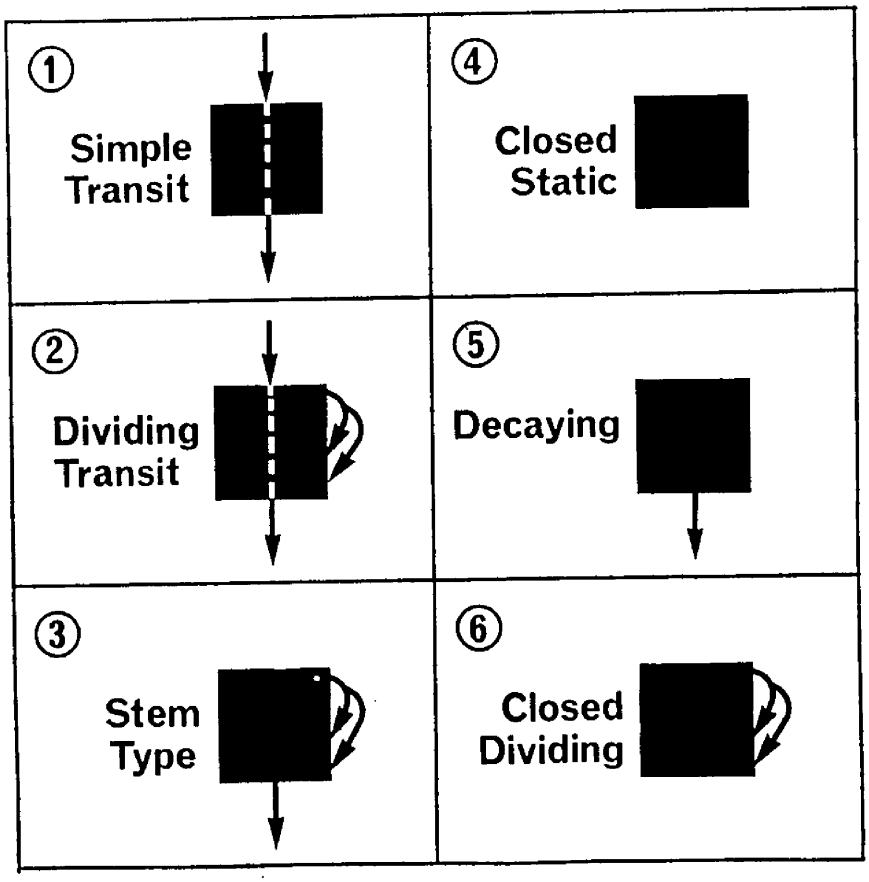
dose-rate and homogeneity of dose. In Section E, the effects of external x-rays on cell survival were remeasured and compared, in the same circumstances, with the effects of iodine-131 and iodine-125.

After the studies in Sections D and E it was then possible to evaluate the radiobiological findings in respect of hormonogenesis and cell proliferation in the rat thyroid as they might apply to radiation effects on normal human thyroid and on thyrotoxic human thyroid. The latter aspect is included as a special section (F) which contains a description of a trial of iodine-125 therapy for thyrotoxicosis.

SECTION D

MEASUREMENT OF THE EFFECTS OF IRRADIATION ON RAT THYROID CELL PROLIFERATION AND D.N.A. SYNTHESIS IN VIVO

CELL POPULATIONS – TYPES



[Modified from Gilbert and Lajtha 1965]

Fig. D-1 Classification of cell populations

Rat thyroid growth induced by a goitrogenic

of Type 6.

Introduction.

The rat thyroid is a suitable model with which to study radiation effects on thyroid cell proliferation in vivo. The model has the advantage of a relatively simple cell population; about 70 per cent of the cells are follicular and about 30 per cent are stromal (Sautler, 1957). There is no migration of cells to or from the gland either normally or when growth is artificially promoted by a goitrogenic challenge. The tissue is thus normally closed and non-dividing, or when goitrogen stimulated which induces cell hypertrophy and hyperplasia it is closed and dividing (Gilbert and Lajtha, 1965). In Fig. D-1 the goitrogenic growth in the thyroid is Type 6.

Ionising irradiations impair the capacity of the rat thyroid to undergo its normal 2 - 3 fold increase in weight in response to a goitrogenic growth stimulus in vivo. The degree of impairment of weight response has been employed as an approximate index of irradiation effects on the reproductive potential of thyroid cells (Doniach 1958, Gibson and Doniach 1967, Philp et. al. 1969, Philp, Crooks, Macgregor and McIntosh 1969). In these latter studies, an assessment was made of changes during growth with and without irradiation due to cell size and number using histological measurements. In the current investigation these studies are complemented by biochemical measurements of nucleic acid synthesis. In addition, irradiation of the follicular and stromal cells have been studied in the present study.

A detailed study of the effects of different doses of

X-irradiation on the thyroid cell population of the normal rat and on the cell population during goitrogenic growth was carried out. The techniques employed included sequential measurement of total thyroid weight, cell density, cell composition, R.N.A. and D.N.A. synthesis using chemical and cell labelling methods. The data are critically discussed with emphasis on how to use the rat thyroid as a radiobiological model with which to study radiation effects on proliferation of differentiated thyroid cells in vivo. X-irradiation was used as a precisely dosed and homogeneous radiation.

Methods and Materials.

X-irradiation.

All the methods are standard; X-irradiation of the rat thyroid was through the ventral neck surface of animals as described in Section B. The conditions were 300 KeV, 20 mA, 23 cms F.S.D., and the dose-rate was 190 rads/min. Control animals were anaesthetised but were not irradiated. All rats were adult male Sprague-Dawley common stock (Tuck and Sons, England).

The rats were sacrificed using coal gas or ether and each freshly dissected thyroid lobe was weighed to 0.1 mg. Thyroid weights were expressed as the mean weight of the two thyroid lobes for the groups specified below.

Cell Density and Composition.

Haematoxylin and eosin sections (5 u) of thyroid were prepared (in a research pathology laboratory) from one lobe and viewed through a squared eye-piece graticule using conventional light microscopy (Watson Microsystem 70). The magnification and

viewing characteristics were kept constant and the total number of cells, follicular and stromal, in fifty fields were counted. Relative cell density was expressed as the average number of thyroid cells of all types per field. In the same fields the follicular cells were distinguished from the stromal cells and the percentage ratio of each was obtained.

Chemical R.N.A. and D.N.A.

The other thyroid lobe was stored at -20°C and the total D.N.A. and R.N.A. estimated exactly as published by Begg, McGirr and Munro (1965) ~~and Flock (1966).~~ These methods are based on perchloric acid extraction and alkali digestion. D.N.A. was measured by the Ceriotti colour reaction and R.N.A. by optical density at 260 u. Mean total D.N.A. per thyroid and mean total R.N.A. per thyroid for the rats in each group were expressed in ug. The D.N.A. control was calf thymus D.N.A. (sigma type 1) and R.N.A. control was purified yeast (sigma type XI).

D.N.A. Synthesis.

When D.N.A. synthesis was to be studied a pulse label of tritiated thymidine (thymidine-6Tn, Radiochemical Centre, Amersham) was given intraperitoneally in a single dose of 0.5 uCi per gram body weight. The specific activity varied from 17.0 to 28.0 Ci/mM. The animals were killed 4 hours after injection and thyroids from each treatment group were pooled. Preliminary nucleic acid extraction was made as described by Begg, McGirr and Munro (1965). The precipitate containing the D.N.A. was digested using 0.6 N Nuclear Chicago Solubiliser Intoluene (Moorhead and MacFarland,

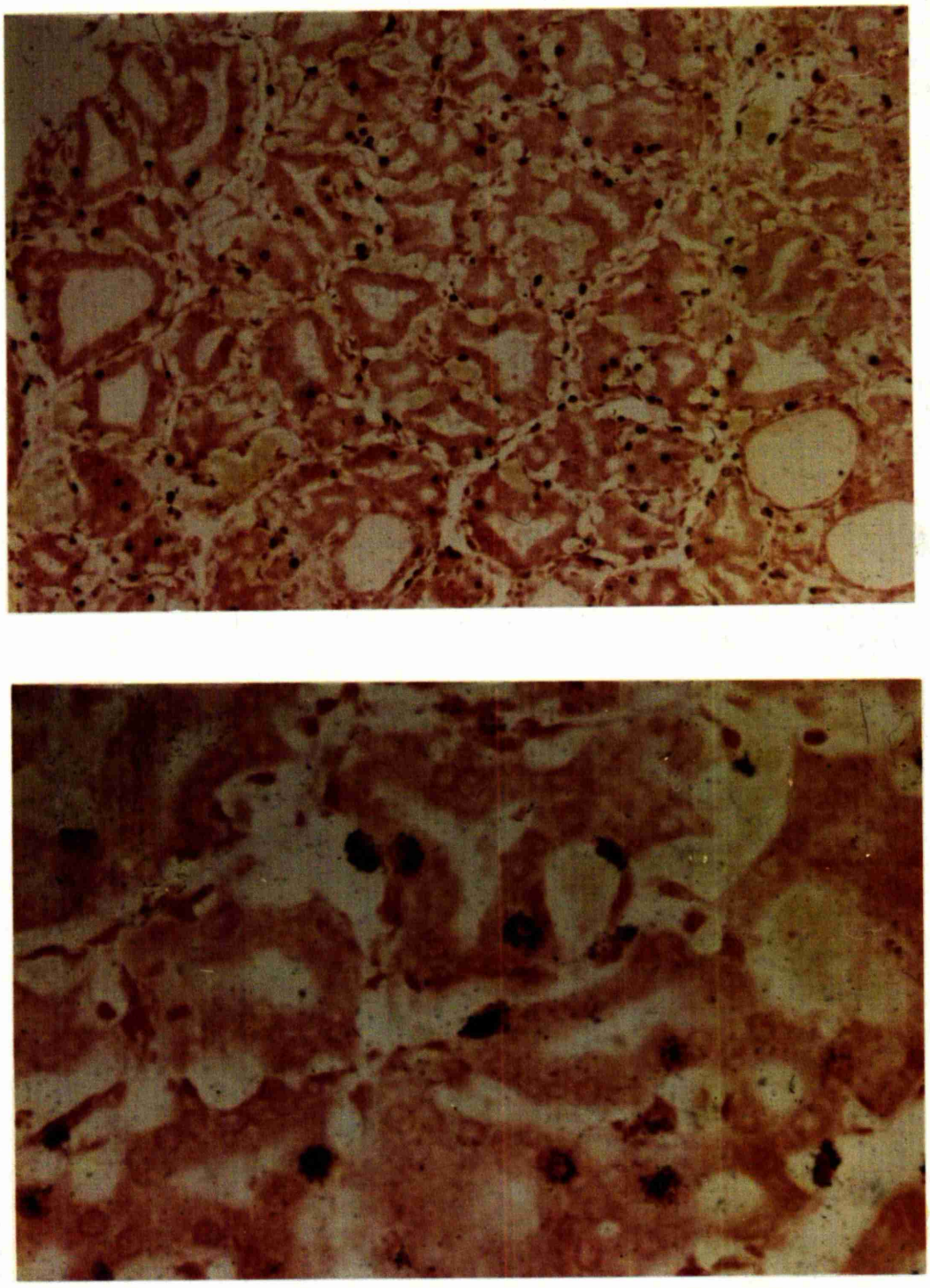
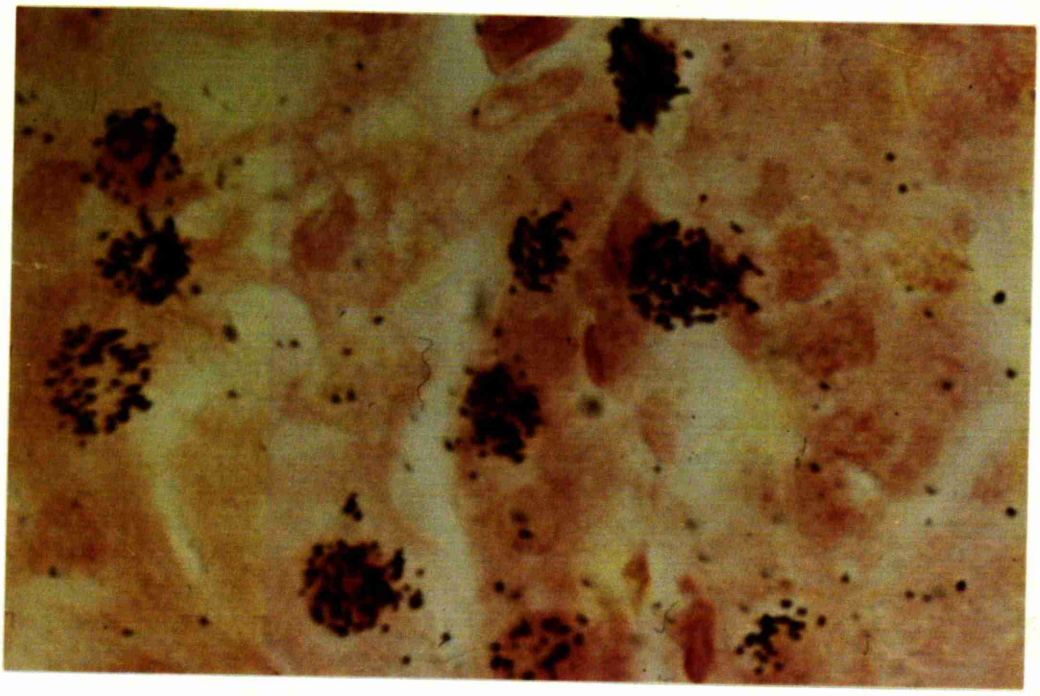


Fig. D-2 Nuclear Emulsion Autoradiographs of Rat Thyroid Cell D.N.A. labelled with Tritiated Thymidine.



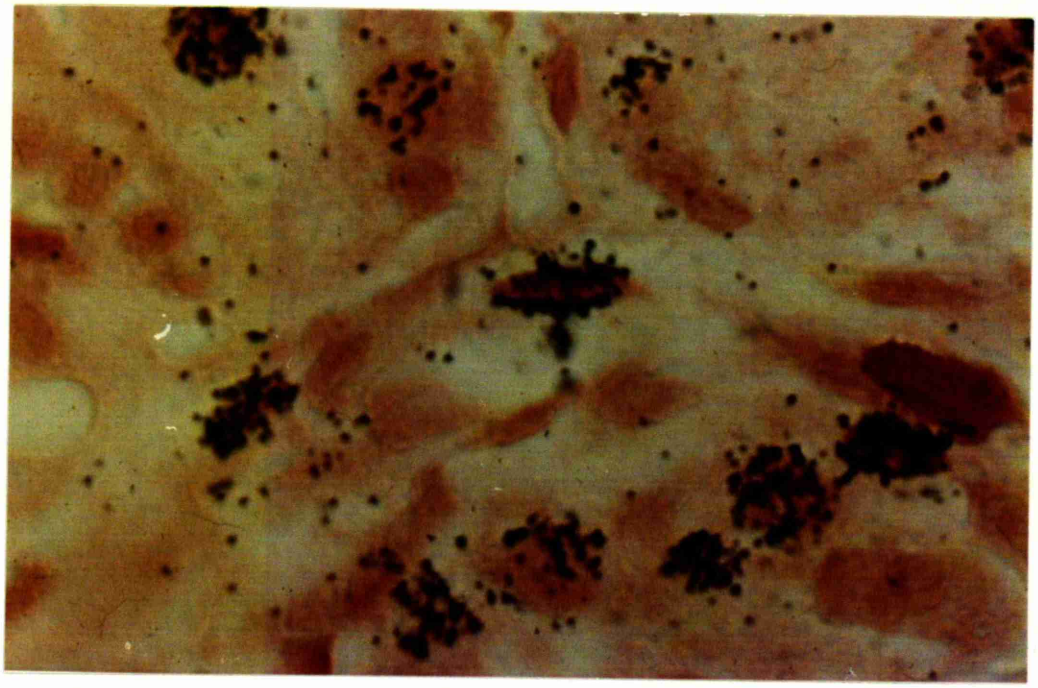


Fig. D-3 Nuclear emulsion autoradiographs of rat thyroid cell D.N.A. labelled with Tritiated Thymidine.

1966, Hansen and Bush 1967). Counting was carried out in the Toluene-P.P.O.-P.O.P.O.P. liquid scintillation system (White, 1968) and expressed as disintegrations per minute (D.P.M.) as described in Section C. Tritiated thymidine incorporation into the nuclei of thyroid cell populations was the mean D.P.M. per whole thyroid for rats in each treatment group as specified below. Chemical D.N.A. was not measured simultaneously with tritiated thymidine studies since it was found in preliminary experiments that the complete chemical procedure resulted in loss of label occuring at stage of alkali digestion.

D.N.A. Labelling Index.

Nuclear emulsion autoradiographs were also prepared using Kodak N.T.B. 2 nuclear emulsion exactly as described by Kopriwa and Leblond (1962). They were exposed at 4°C for three to four weeks, stained with neutral red, mounted and viewed using the microscopic characteristics described above for the determination of cell density. Fifty fields were surveyed from each treatment group and the number of cells labelled were counted. Labelled cells were those with a minimum of 20 grains. Mirror sections not used for autoradiography were stained with haematoxylin and eosin and the total number of cells in the 50 fields were determined. The labelling index was expressed as the number of labelled cells per 5,000 cells. In all cases the labelled cells were randomly distributed throughout the tissue sections. Examples of D.N.A. labelled cells, follicular and stromal are shwon in Figs. D-2 & D-3

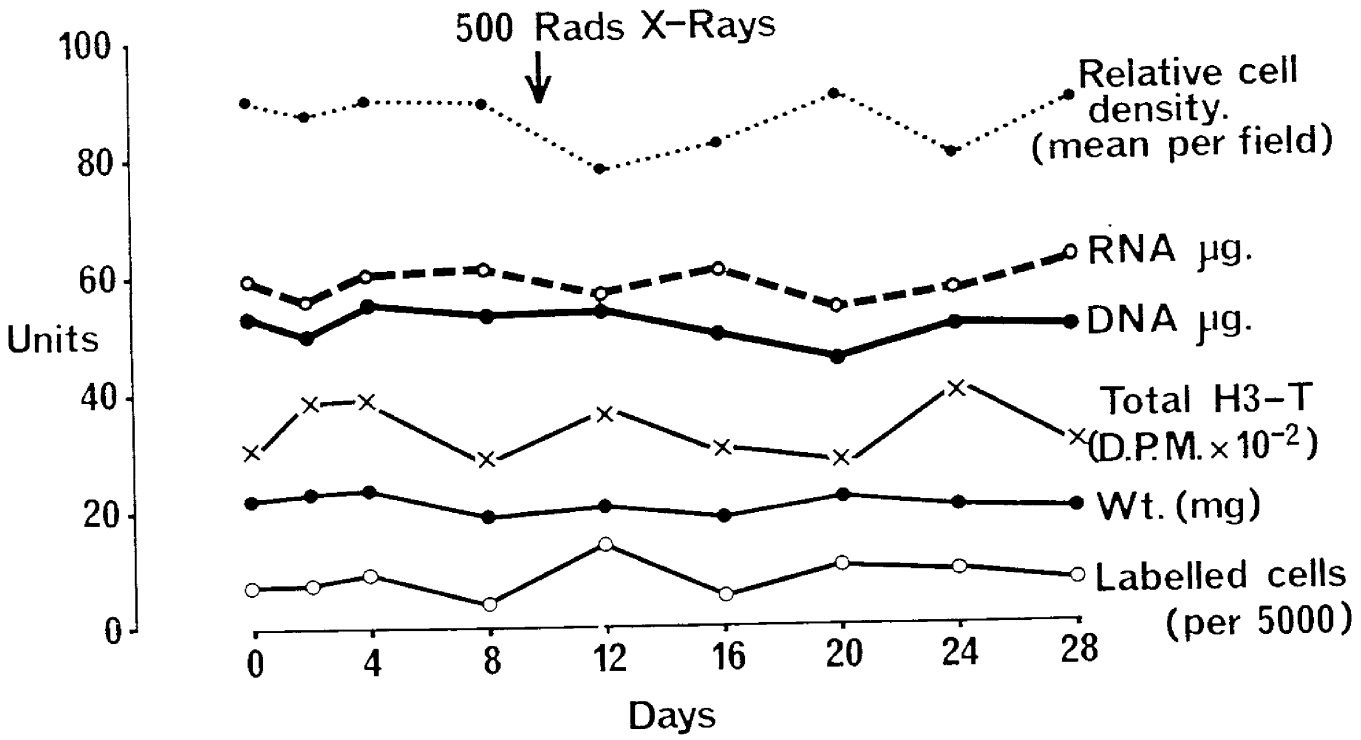


Fig. D-4 Cell proliferation and D.N.A. synthesis in normal rat thyroid: Sequential measurements on normal rat thyroid before and after single dose of X-rays (500 rads). Values are means from 6 animals per sacrifice.

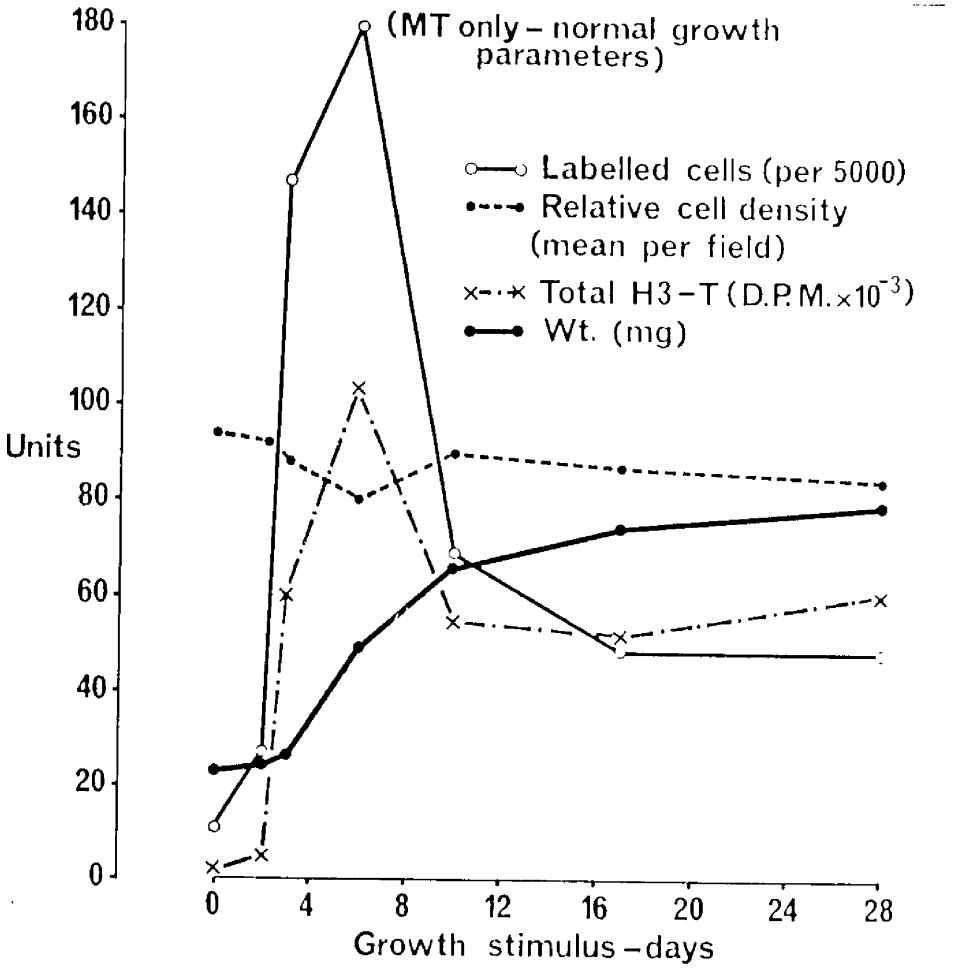


Fig. D-5 Cell proliferation and D.N.A. synthesis during goitrogenic challenge.

No irradiation:

Sequential measurements on rat thyroid before (0 days) and at intervals during continuous promotion of thyroid growth using 0.1 per cent methylthiouracil and low iodine diet for 28 days (goitrogenic challenge). Values are means from 5 animals per sacrifice. To be compared with Fig. D-4.

Experiments, Results and Interpretations.

1. Effects of X-rays on Normal Thyroid. Experiment 1, Fig. D-4.

Thyroid weight, cell density, chemical D.N.A. and R.N.A. tritiated thymidine incorporation and labelling index were determined at regular intervals before and after a single dose of 500 rads, a large dose in radiobiological terms. Groups of 6 rats were sacrificed at each of the 9 times indicated.

The data (Fig. D-4) demonstrate that in normal adult rats there is virtually no cell proliferation and that a single x-ray dose of 500 rads induces no changes; the potentially most sensitive indices of normal cell proliferation, tritiated thymidine incorporation and labelling index, both remained very low throughout the period of observation.

It can be concluded that the adult rat thyroid cell population is stationary in phase G_0 or G_1 (Gilbert and Lajtha 1965) and that X-irradiation at a dose of 500 rads does not produce observable change. The next experiments describe the detailed changes in cell proliferation and D.N.A. synthesis brought about by a goitrogenic challenge without irradiation; those provide control data for reference when the thyroids were X-irradiated and then cell proliferation was promoted by the goitrogenic challenge.

2. Effects of a Goitrogenic Challenge on Unirradiated Thyroid. Figs. D-5 to D-10.

Thyroid growth was artificially stimulated by providing 0.1 per cent aqueous methylthiouracil (4 methyl - 2 thiouracil B.D.H. Labs) as drinking water and diet of low iodine content.

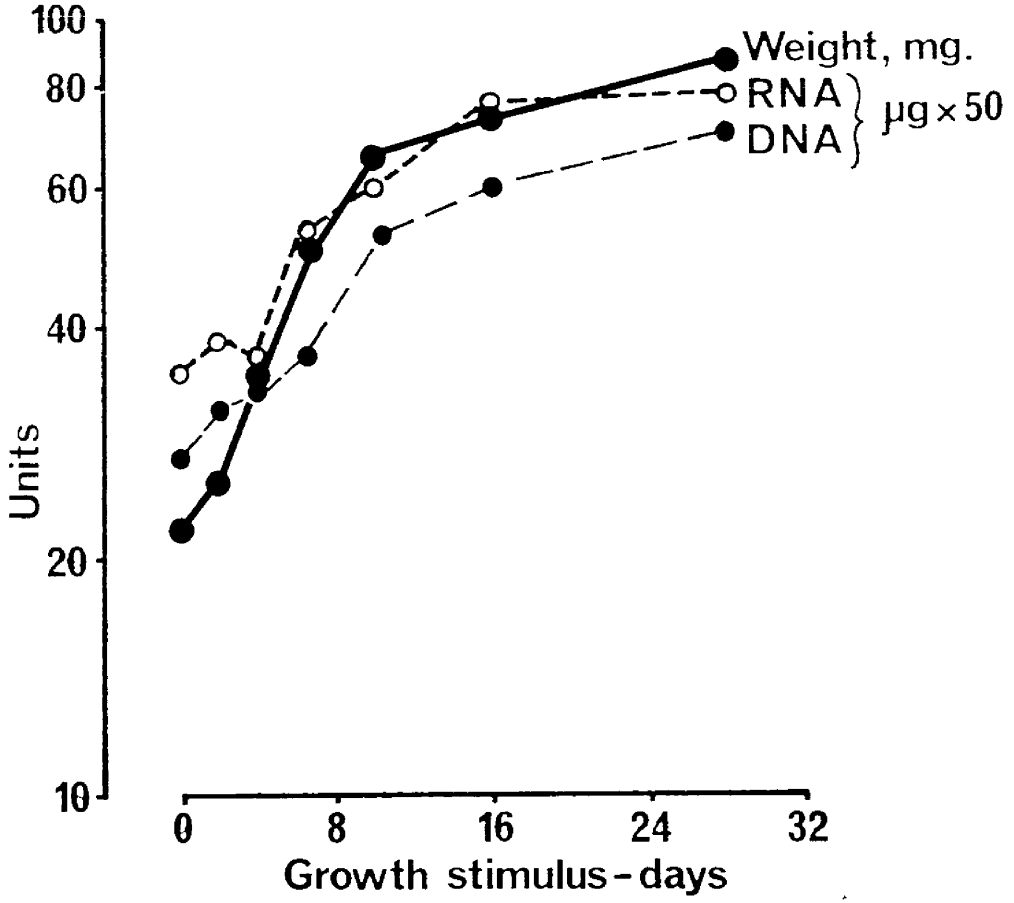


Fig. D-6 Cell proliferation and R.N.A./D.N.A. synthesis during goitrogenic challenge - No irradiation.

Data are mean thyroid weight and chemical R.N.A./D.N.A. - 5 animals per group.

Fig. D-7 Left - normal rat thyroid (attached to thyroid cartilage and upper trachea) right - rat thyroid after 28 day goitrogenic growth stimulus - no irradiation.

Groups of 5 rats were sacrificed at each of the 7 times indicated. During this interval the drug interrupts hormonogenesis and is stimulated by T.S.H. (Brodish 1968).

The data (Fig. D-5) show that before the goitrogenic challenge the labelling index and tritiated thymidine incorporation were very low, reconfirming that under normal conditions very few of the cells were in generative cycle. Within 2 days of administration of the goitrogen, however, both the labelling index and the tritiated thymidine incorporation increased markedly, then rose to high levels corresponding with the rapid growth of the thyroid till the 8th day, and finally fell as growth slowed. Cell density dropped toward a small dip about day 6 followed by a slow fall, but the total decline was not substantial.

3. Experiment 3, Fig. D-6.

In this experiment thyroid weight and chemical D.N.A. and R.N.A. were measured before andduring a goitrogenic challenge. Seven groups of 5 rats were used. It should be noted that the ordinate scale in Fig. D-6 is logarithmic. The graphs showed that D.N.A. and R.N.A. both rose in parallel with thyroid weight, but not quite to the same extent. Fig. D-7 shows a rat thyroid before goitrogen and a rat thyroid after a 28 day goitrogenic growth stimulus.

4. Experiment 4, Fig. D-8.

Groups of 3 rats were sacrificed at times shown before and during goitrogen stimulation. Thyroid weight and stromal cells (including labelled) as a percentage of all cells were

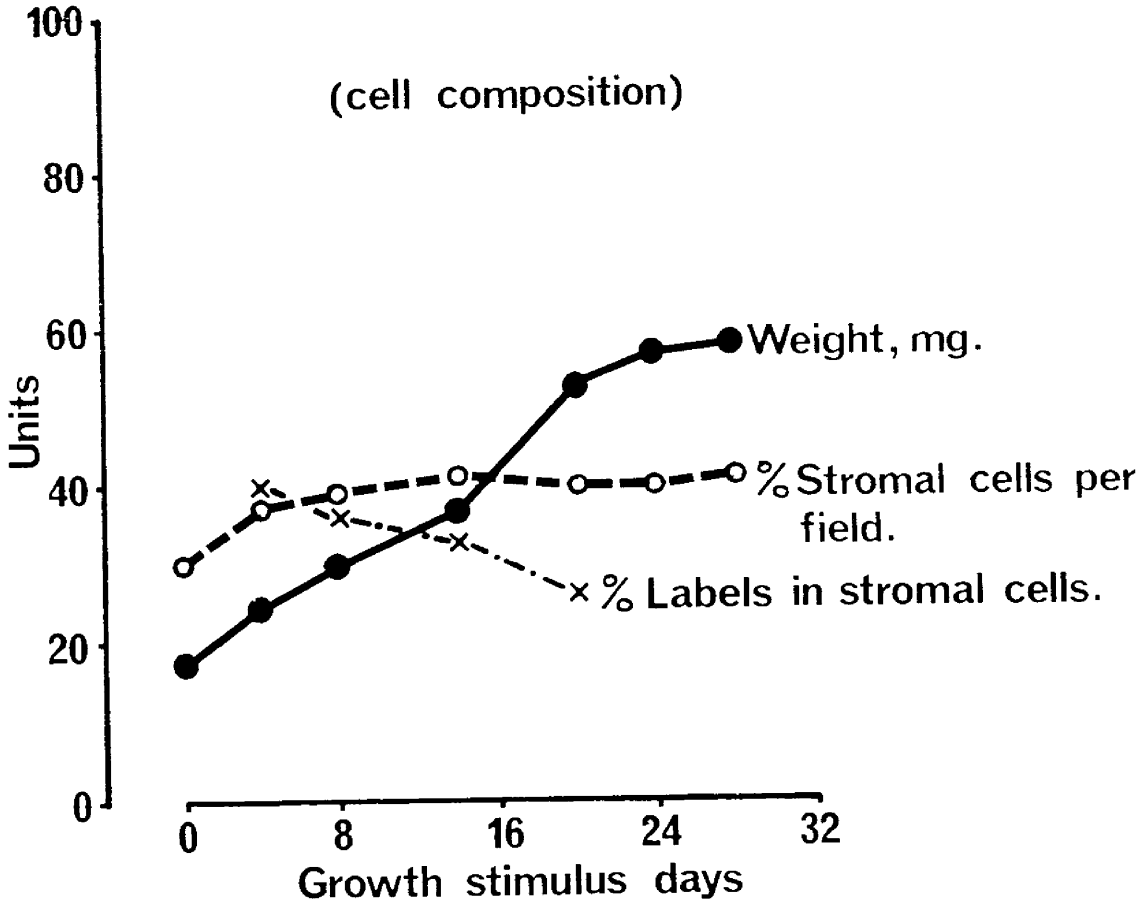


Fig. 5-3 Proliferation of stromal and follicular cells during oestrogenic challenge - No irradiation.

Values are cells as percentage of total based on cell counts on 50 fields from 3 rats per condition; per cent stromal cells is percentage of all cells; per cent labels in stromal cells is percentage of all labelled cells.

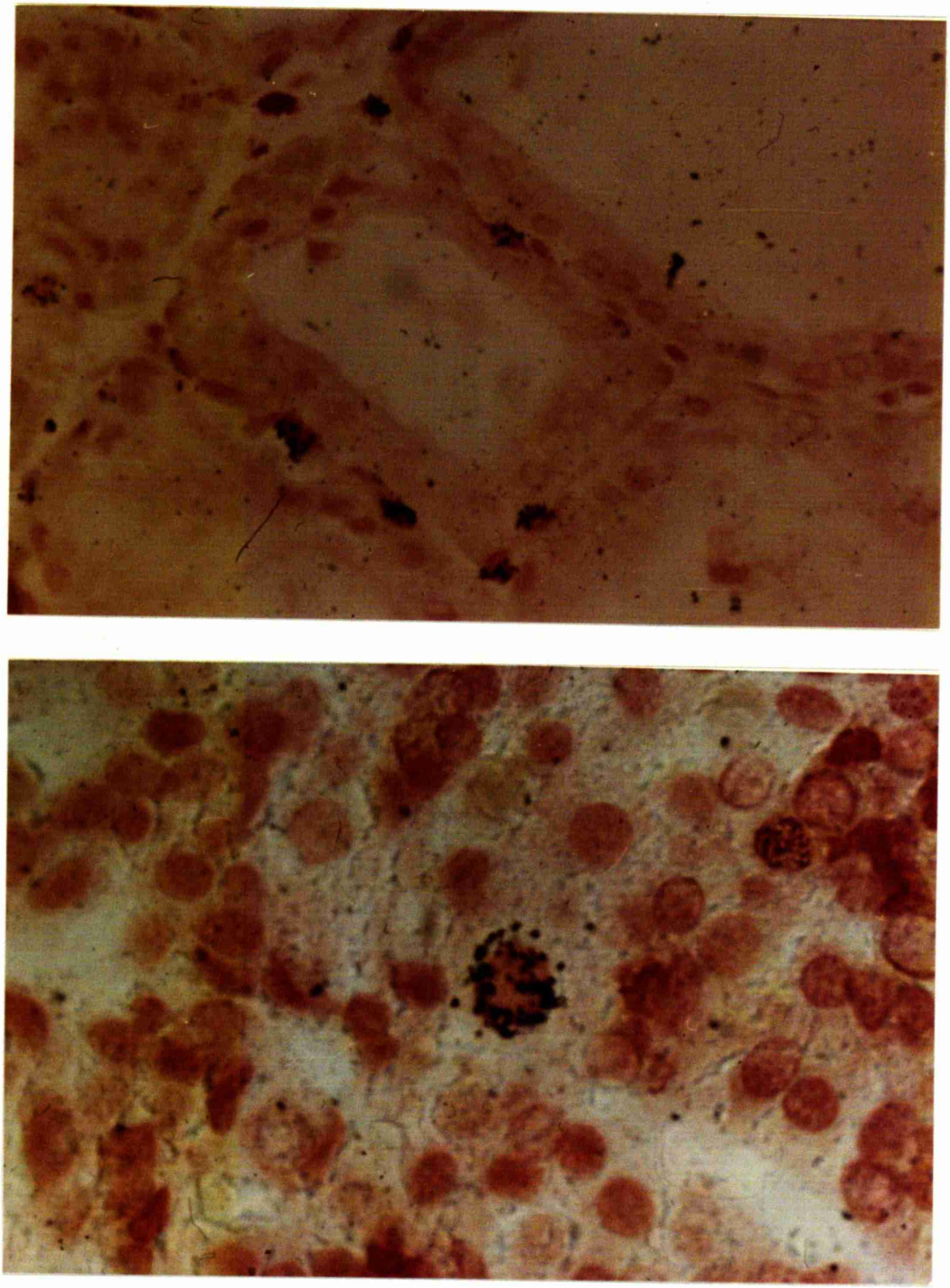


Fig. D-9 Labelled rat thyroid stromal cells (top) and a labelled rat pituitary cell (bottom) during goitrogenic challenge.

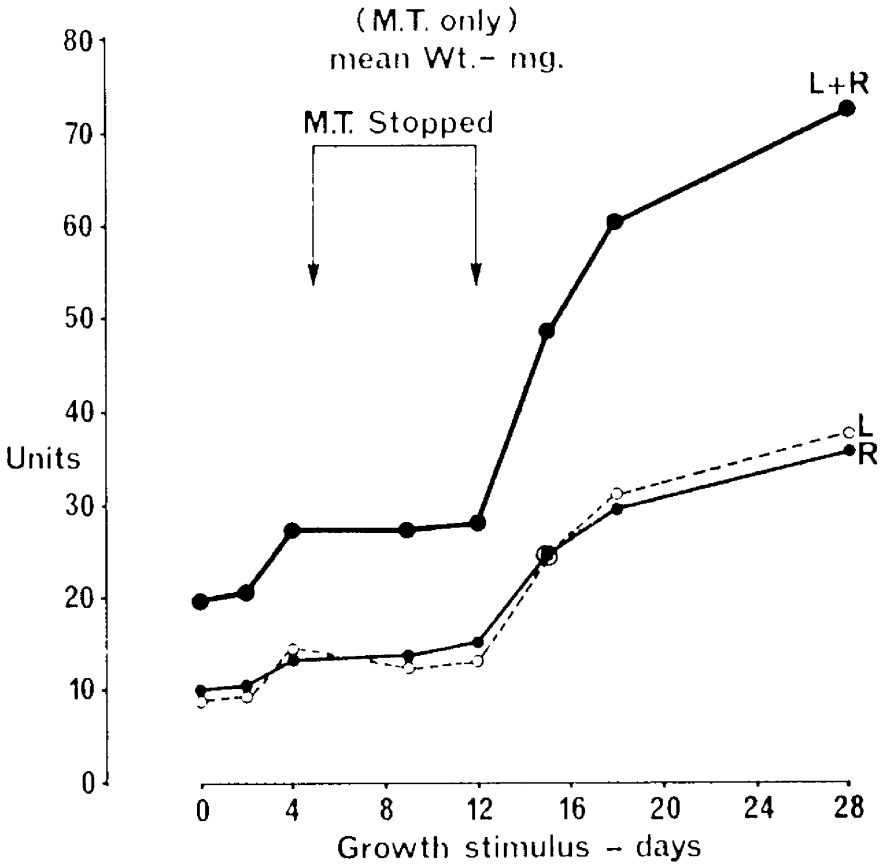


Fig. D-10 Thyroid weight change pattern during temporary cessation of goitrogenic challenge - no irradiation. Goitrogen stopped from day 5 to day 12. Values are means from 5 animals per sacrifice.

measured. The Fig. D-8 shows that the proportion of stromal cells increased from 30 per cent to 42 per cent. (Fig. D-9 shows labelled stromal cells and a labelled pituitary cell. Goitrogenesis brings about cell division in pituitary too).

5. Experiment 5, Fig. D-10.

In this experiment groups of 5 rats were commenced at day 0 on the goitrogen which was continued for 5 days. From the 5th to the 12th day, however, the goitrogen was removed and the rats took water and standard diet only. At the 12th day the same goitrogen was recommenced and continued up to the 28th day. The points shown are the mean of thyroid total weight and the means of the weights of the left and right lobes respectively (L= left lobe and R = right lobe).

This experiment (Fig. D-10) showed that a temporary cessation of the goitrogenic stimulus results in an immediate stop to the thyroid mass increase, but resumption of the stimulus after an interval of 7 days produces a continuation of the mass increase, without an additional lag phase, proceeding as before. In this context, it has been said that if one thyroid lobe is removed at the time when weight has reached a maximum plateau (28 days) the remaining lobe does not double its weight but remains unchanged (Doniach 1968).

6. Comment on Goitrogen Induced Thyroid Growth Without Irradiation (Control).

In considering the actual sequence of events during the weight increase due to goitrogenic stimulation in the absence of

irradiation a number of points of evidence must be linked. The initial low labelling index and low tritiated thymidine incorporation showed that cell turnover was very low before the goitrogen (Fig. D-4). The high peaks in both of these indices during goitrogenic stimulation corresponded to the maximum rate of increase of both weight and chemical D.N.A. (Figs. D-5 and D-6). The low labelling index found as the growth curve flattens off showed that the number of cells synthesising D.N.A. simultaneously was much reduced as the growth slowed. The near parallel increases in chemical D.N.A. and R.N.A. and mass (Fig. D-6) show that on average the mean number of cells increases concomitantly with total cell mass and thyroid weight. Follicular cells do increase in size before division during goitrogenic hyperplasia but this change appears to be offset by loss of colloid. The net result is that cell density remains relatively unaltered during goitrogenic growth as shown in Fig. D-5. These conclusions were also reached recently by Philp et. al. 1969 and in general by Doniach (1960).

Goitrogen induced rat thyroid growth appears therefore to be a well regulated but special type of growth. The picture which can be drawn is of the majority of the thyroid cells responding, after a short lag phase during which hormone stores (colloid) are depleted, to the goitrogen by moving into active generative cycling. Synthesis of D.N.A. and cell division appear to proceed in balance and maintain the normality of the cells and most of the organ growth (weight) is due to the increase in cell

numbers. The number of cells synthesising D.N.A. and dividing soon falls steeply, however, and the rate of growth slows to approach an apparent maximum asymptotically.

It might be postulated that this limitation of growth arises from extrathyroidal control, nutritional deficiences in the goitrous state, or to factors intrinsic to the thyroid cells themselves. It is unlikely that extrathyroidal factors exist which are specific to only one thyroid lobe (Doniach 1968). The evidence of Experiment 4 showing a greater proportional rise in stromal cells (Fig. D-8) is against the postulate of nutritional deficiencies. It would appear, therefore, that it is the thyroid cells which have an inherent limited divisional capacity seen as a ceiling or plateau limit to organ growth despite continued stimulation and adequate nutrition.

Although most of the weight increase is due to an increase in cell numbers, a significant part must arise from an increase in the average size of the cells. The average contribution made by cell hypertrophy would appear to be the 20 per cent by which the total D.N.A., an index of cell number, failed to rise proportionately to the total weight (Fig. D-6). The proportional contribution to thyroid mass made by non-cellular structure other than colloid during goitrogenic growth is small (Santler 1957, Chow and Woodbury 1965).

In the light of the comments made above concerning the cellular events occurring during the 28 day goitrogenic stimulation

In the absence of irradiation the experiments using irradiation can now be presented. In these, latent damage was first produced by single doses of x-rays to normal rat thyroid then after an interval of 4 weeks the effects of the damage to the cell population was measured. It has previously been shown that latent radiation injury to thyroid cell reproductive integrity is permanent (Davis, Doyle, Rahman and Dalton 1969).

4. Effects of a Goitrogenic Challenge on Irradiated Thyroid Experiment 6, Figs. D-11 to D-15.

Single x-ray doses of 100 rads, 500 rads and 1,000 rads were given to groups of rats. All animals were commenced on the goitrogenic regime 4 weeks after irradiation. Sub-groups of 5 animals from each x-ray dose group were sacrificed at the times indicated in Fig. D-11, that is just before and up to 28 days after the start of goitrogenic stimulation.

The effects of these various x-ray doses on the response to the goitrogen as revealed by thyroid weight, cell density AND TYPE total unlabeled thymidine incorporation, labelling index ~~and thyroid cell composition~~ are shown in Figs. D-11 to D-15 respectively. Each figure is discussed separately and includes for comparative reference the data from Experiment 1 to 4 (Figs. D-1, D-5, D-6 and D-9) which is from non-irradiated thyroids subjected to the same goitrogenic procedure (control).

1. Effects of Irradiation on Thyroid Weight - Fig. D-11.

The greatest effect was seen after 1,000 rads. With this dose the thyroid weight increased at a normal rate for newly

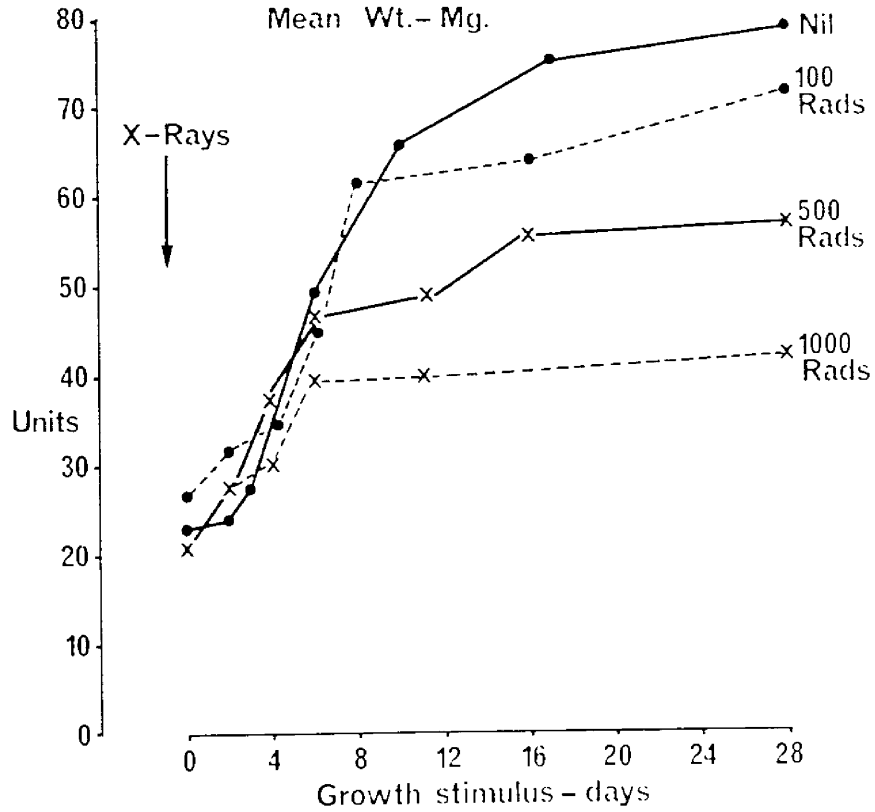


Fig. D-11 Effects of X-irradiation on thyroid growth during goitrogenic growth stimulus – values are mean thyroid weights from 5 rats.

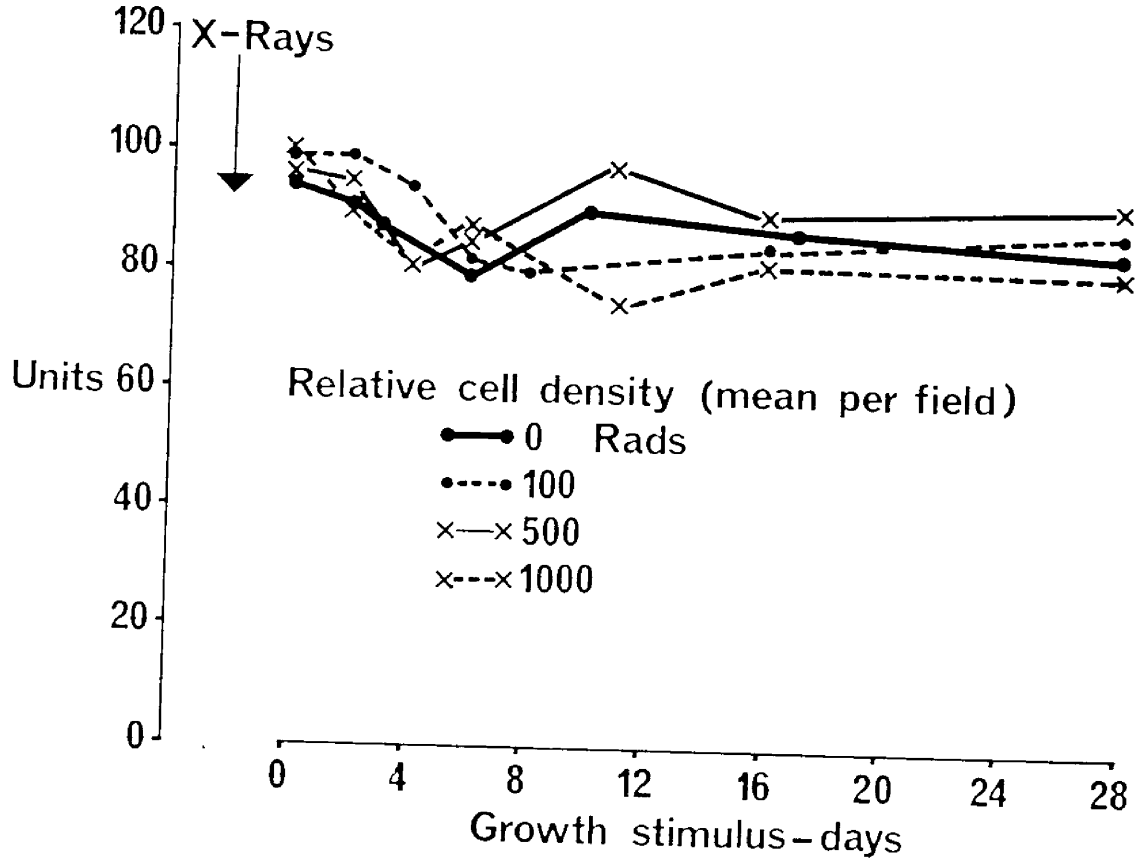


Fig. D-12 Effects of X-irradiation on total thyroid cell density during goitrogenic growth stimulus - values are mean cell density in 50 fields from 5 thyroids.

6 days but then growth stopped. With 500 rads weight increased normally for 6 days and after 6 days growth was slow but not halted. 100 rads did not produce an effect significantly different from the unirradiated response, although a slight impairment of growth was discerned. Thus, for all three radiation doses the thyroid weight increased normally during early growth promotion but at about the 6th day of growth the weight curves in rats given 500 rads and 1,000 rads x-rays plateaued and growth was thereafter much decreased, the degree of final impairment (weight at 28 days) being x-ray dose determined. It would appear, therefore, that the mode of action of X-irradiation in limiting the ultimate capacity of the thyroid to grow is linked to the later stages of growth.

2. Effects of X-irradiation on Cell Density - Fig. D-12.

There was no significant difference between the sequential patterns of cell density in the irradiated thyroids and the controls, both showing a slight fall during growth promotion. This shows that, compared to normal, the ratio of cell number to cell size and non-cellular structure is unaffected by irradiation.

3. Effects of X-irradiation on Stromal Cell Percentage - Fig. D-13.

The increase in stromal cells (percentage of total cells) during goitrogenic challenge was greater after 500 rads and 1,000 rads than after 100 rads or no irradiation. This effect may be due to differential x-ray impairing effects on cell proliferation within the two compartments, the stromal cells being more radio-resistant than the follicular cells, or it might arise because stromal cells

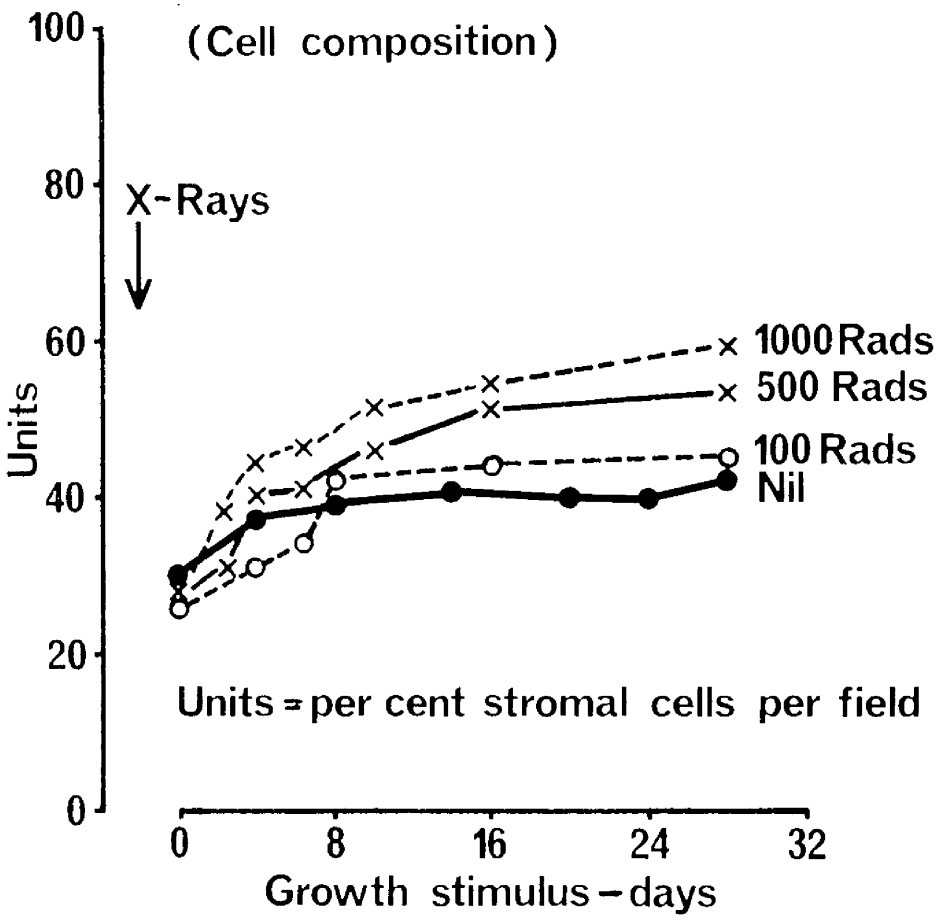


Fig. D-13. Effects of X-irradiation on proliferation of stromal and follicular cells during goitrogenic growth stimulus. Values are per cent total cells based on cell counts in 50 fields from 5 thyroids.

have the stimulating action of tissue injury added to that of the goitrogenic challenge.

4. Effects of X-irradiation on Total Tritiated Thymidine Incorporation - Fig. D-14.

The pattern of total gland tritiated thymidine (H3-T) incorporation after a dose of 100 rads was not significantly different from that of the non-irradiated controls. Since, however, total H3-T uptakes, in the context of these investigations, are indices of average rates of D.N.A. synthesis over all thyroid cells, a lack of effect of x-rays is not incompatible with some sublethal change in detail within the cells as will be discussed below. The measurements for 500 rads showed a considerably higher peak in D.N.A. synthesis than normal between 4 and 8 days, the time when the most vigorous increase in cell numbers should have taken place (as judged from Fig. D-11) and the 1,000 rad peak was higher still.

These increased rates of D.N.A. synthesis after both the higher x-ray doses must mean that the total amount of D.N.A. synthesised in the intermediate period of goitrogenic growth is greater than normal. This is, however, approximately balanced by the lower rates and resulting lower total amounts of D.N.A. synthesised at the last stages of growth (Fig. D-14). Hence the total amounts of D.N.A. synthesised do not appear to be greatly affected by X-irradiation but the aggregated synthesis seems to be performed in a shorter time. The increased total D.N.A.

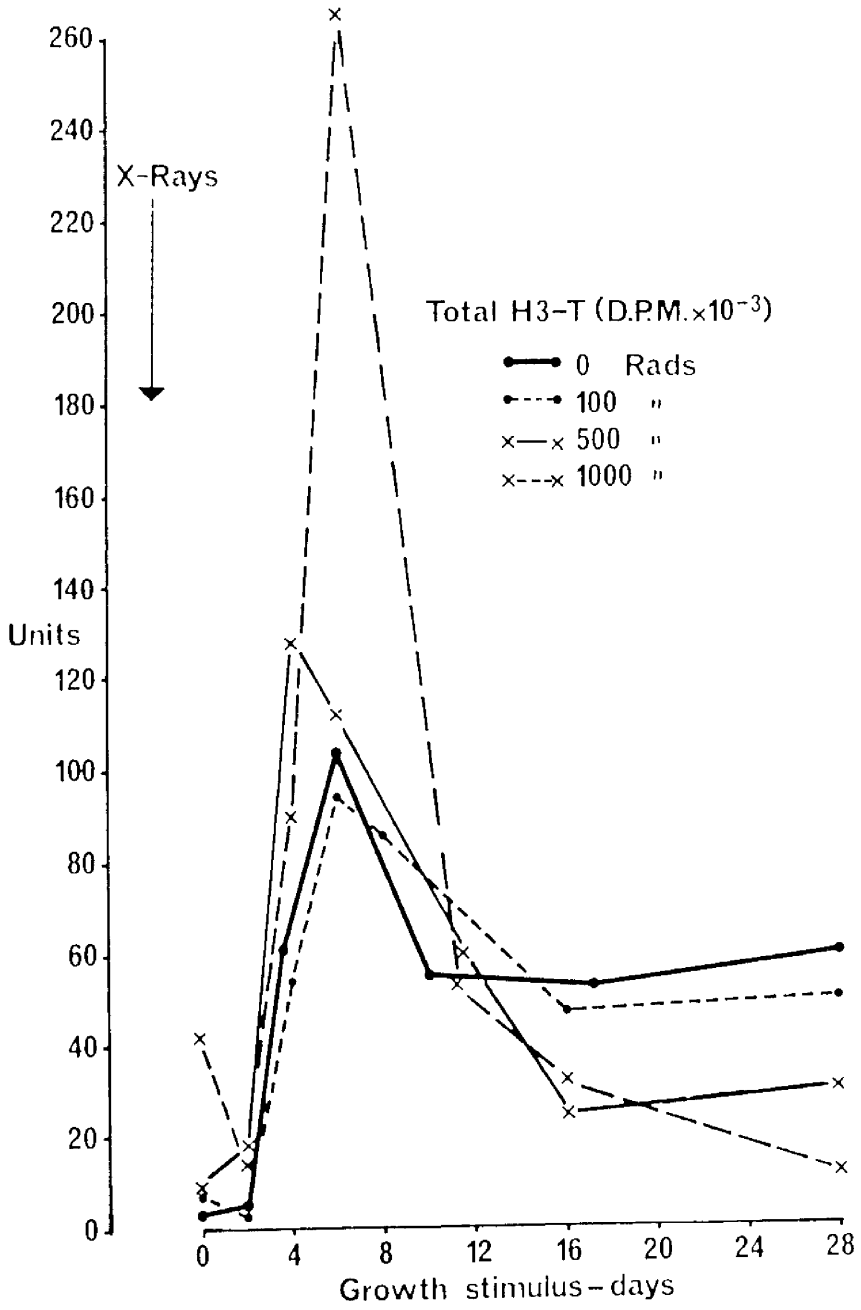


Fig. D-14 Effects of X-irradiation on total thyroid incorporation of tritiated thymidine into D.N.A. (total H3-T) during goitrogenic growth stimulus. Values are D.P.M. from 5 pooled thyroids.

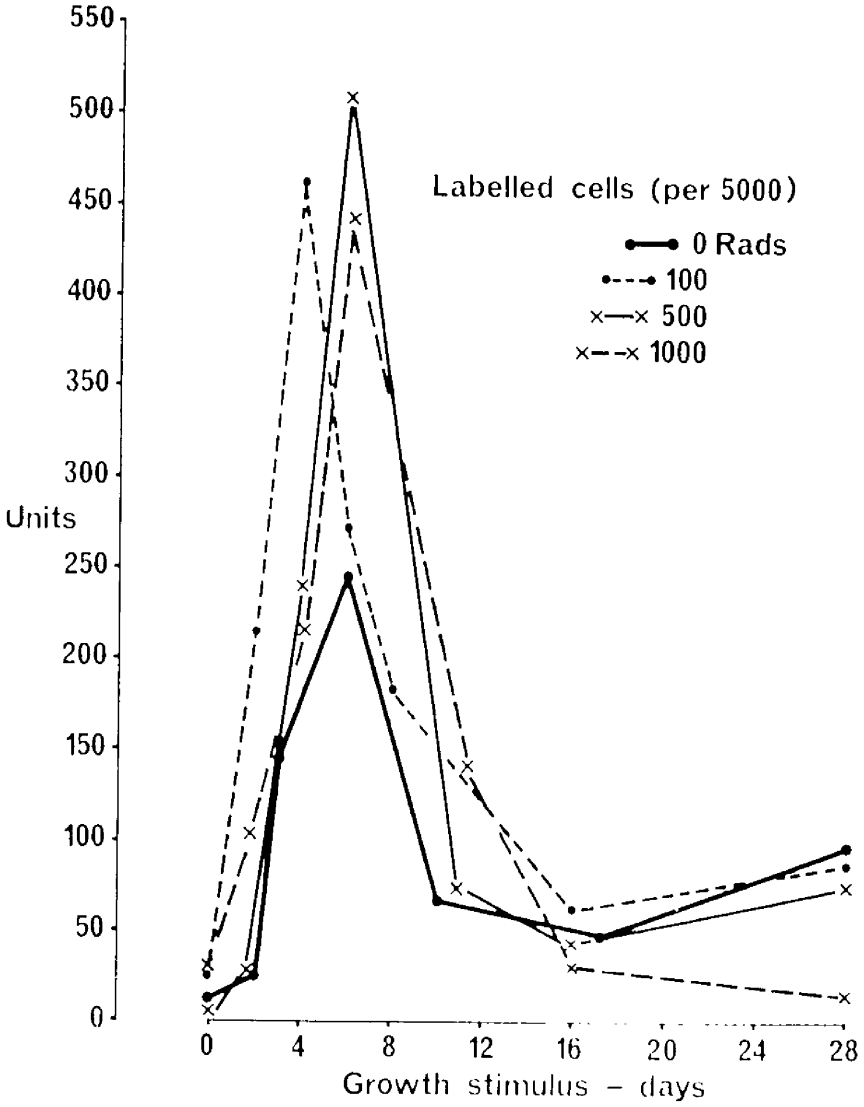


Fig. D-15 Effects of X-irradiation on labelled thyroid cells (H3-T autoradiographs) per 5,000 total cells during goitrogenic growth stimulus. Values are from 5 thyroids.

synthesised in the first part of the goitrogenic response following 500 and 1,000 rads could arise through three different mechanisms: more cells might be synthesising D.N.A. at a normal rate or a normal number of cells could be synthesising D.N.A. faster than normal, or fewer cells could be synthesising very much more D.N.A. and rapidly. These possibilities were examined below in the light of the labelling index data.

5. Effects of X-irradiation on Labelling Index - Fig. D-15.

In irradiated thyroid very large peaks in labelling index (labelled cells per 5,000 cells) occurred at the same time as the unirradiated peak and after 1,000 rads and 500 rads the labelling index was twice as high as in unirradiated gland (Fig. D-15). The post-irradiation values reached 500 per 5,000 cells compared to 250 for non-irradiated thyroid. These data are taken to mean that throughout the period of most vigorous cell D.N.A. synthesis, twice as many irradiated cells, as control cells, were in active D.N.A. synthesis, and since they synthesised about twice as much total D.N.A. as non-irradiated gland (Fig. D-14) the mean rates must have been approximately normal. After this phase of high labelling (Fig. D-15) the labelling indices fell, and after 1,000 rads the fall was to below normal levels. Following 100 rads the labelling indices reach as high an abnormal peak as after 500 rads or 1,000 rads. This contrasts with the relative normality of the total tritiated thymidine uptake (Fig. D-14). Thus after 100 rads, although total D.N.A. synthesis rates were normal, the

number of cells involved during rapid thyroid growth was about doubled. A possible explanation for this is either that repair of D.N.A. is important or that a somewhat longer and slower S-phase within a cell cycle of unchanged length results from the moderate sublethal damage produced by the 100 rads; sublethal means here, a subtle change in the cell generative cycle but not sufficient to alter viability or reproductive capacity. The subtle alterations in pattern of D.N.A. synthesis after 100 rads (Figs. D-14 and D-15) which did not significant impair goitrogenic growth (Fig. D-11) contrasted with the effects of 500 and 1,000 rads which were decisive.

The effect patterns which emerge with these higher doses of X-irradiation and goitrogenic growth promotion over 28 days (Figs. D-11, D-14 and D-15) are thus of an apparently normal initiation of growth and D.N.A. synthesis in the thyroid but the number of cells which are ultimately able to divide is decreased as a result of the irradiation. It would seem that the large number of cells which do not pass into normal mitosis can make D.N.A. As a consequence, although growth is impaired or arrested at the phase of maximum potential increase (Fig. D-11), the total uptake of tritiated thymidine and the proportion of cells labelled is higher than normal (Figs D-14 and D-15). This interpretation is also consistent with the quick fall in total D.N.A. synthesis this probably showing that the number of cells involved after thyroid growth has ceased prematurely falls off sharply.

Other investigators have incidently noted increased uptakes of tritiated thymidine after irradiating rat thyroid and giving iodine deficiency as a goitrogenic stimulus (Dobyns, Rudd and Sanders, 1967). Like them we think the increased cell D.N.A. synthesis is the radiobiochemical counterpart of the large abnormal nuclei noted in morphological preparations of human thyroid following therapeutic doses of irradiation (Dobyns and Didtschenko, 1961, Dobyns and Robinson 1968). These data (Figs. D-14 and D-15) show a high level of apparent abortive D.N.A. synthesis in irradiated cells when they are stimulated into generative cycling. This could also be the radiobiochemical equivalent of chromosome breaks and their repair noted by other investigators examining mammalian thyroid irradiated in vivo (Moore and Colvin 1968, Speight, Baba and Wilson 1968). Increased D.N.A. synthesis in other mammalian cells after their irradiation in vivo and during induced cycling has also been recently reported by Smets (1968) and Watanabe and Okada (1968).

6. Summary of Subsidiary Validation Experiments - Figs D-16, D-17 and D-18.

In order to establish the radiobiological significance of the above observations and exclude artefacts, a number of subsidiary validation experiments were carried out. These will be reported only briefly and their relevance to present investigations summarised.

The possibility was considered that the effects of the

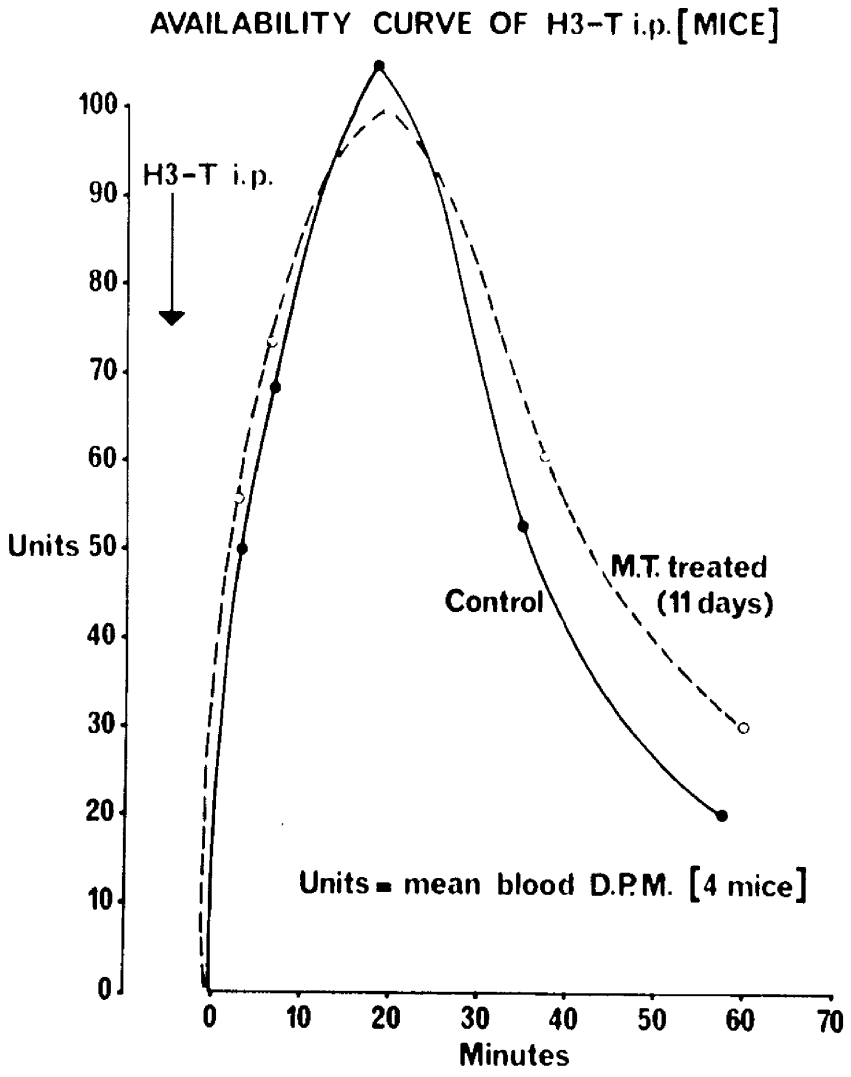


Fig. D-16 Mouse blood tritiated thymidine (D.P.M.) curves after a single intraperitoneal dose (50 uc). Comparison of curve in normal and methylthiouracil treated mice - values are means from same 4 mice.

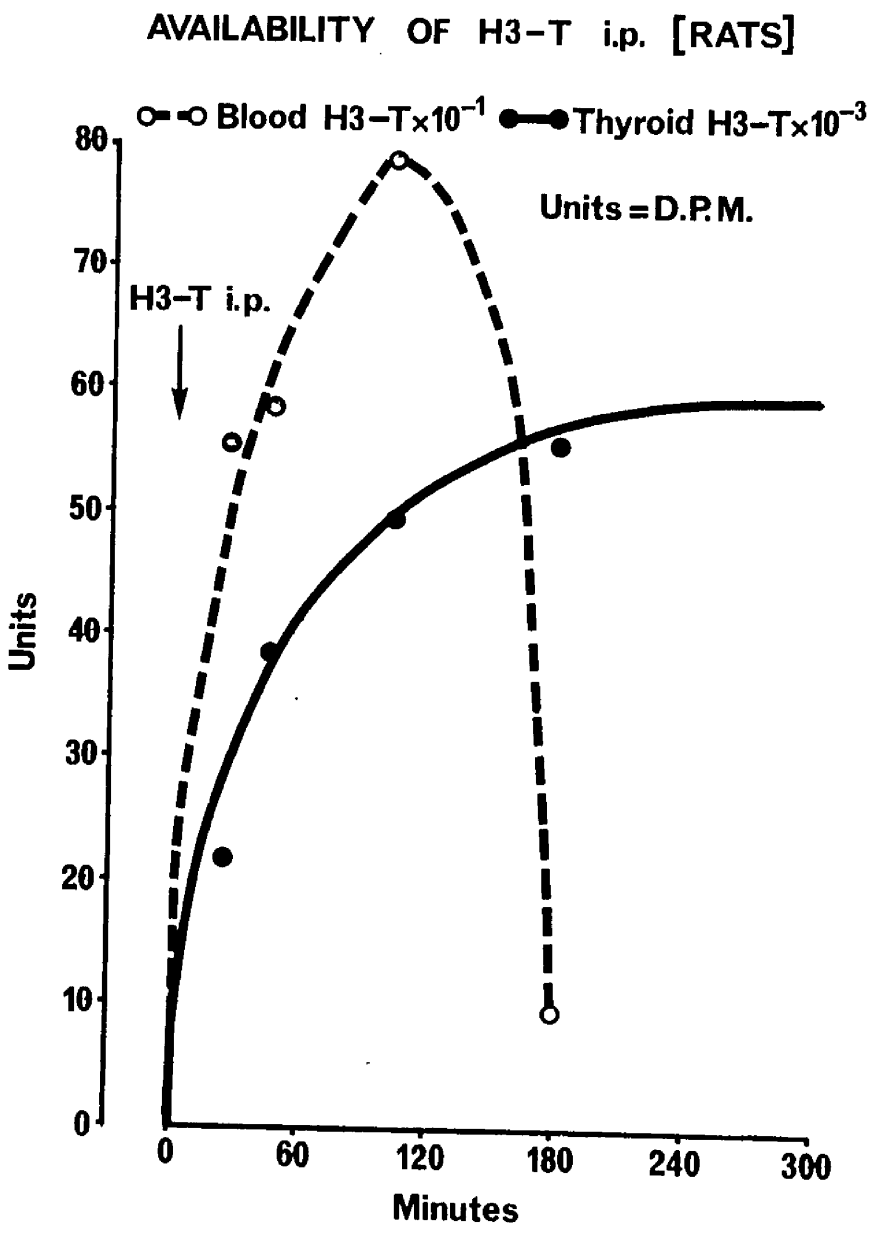


Fig. D-17 Rat thyroid cell D.N.A. tritiated thymidine (D.P.M.) and heart blood tritiated thymidine (D.P.M.) sequentially after a single intraperitoneal dose (0.5 uc/gram). Values are means from [illegible] different pairs.

goitrogenic challenge on tritiated thymidine incorporation and labelling indices might have been an artefact produced by some peculiarity of the drug methylthiouracil. Such a drug effect might have been produced, for example, by a change in the availability time of the tritiated thymidine through a systemic effect of methylthiouracil on body or intrathyroidal thymidine distribution and disposal. This possibility was excluded with three experiments (Figs. D-16, D-17 and D-18).

In mice given tritiated thymidine intraperitoneally the retro-orbital blood tritium radioactivity time curve determined by the method of Hansen and Bush (1967) was the same as that of untreated control mice (Fig. D-16). In rats, the sequential changes in thyroid D.N.A. incorporation of tritiated thymidine measured up to 6 hours after a single administration of tritiated thymidine to methylthiouracil primed animals showed increasing uptake till a plateau commenced at 4 hours after thymidine administration and the thyroid uptake-time curve co-related with the heart blood tritiated thymidine-time curve (Fig. D-17). Another experiment demonstrated that within the limits of specific activity used (17.0 to 28.0 Ci/mM), the rat thyroid incorporation of the tritiated thymidine showed little variation and this applied whether rapid thyroid growth was induced by absolute iodine deficiency or by methylthiouracil (Fig. D-18). This appeared to exclude a possible pharmacological effect of the drug on the thyroid itself.

These studies appear to prove that the changes in

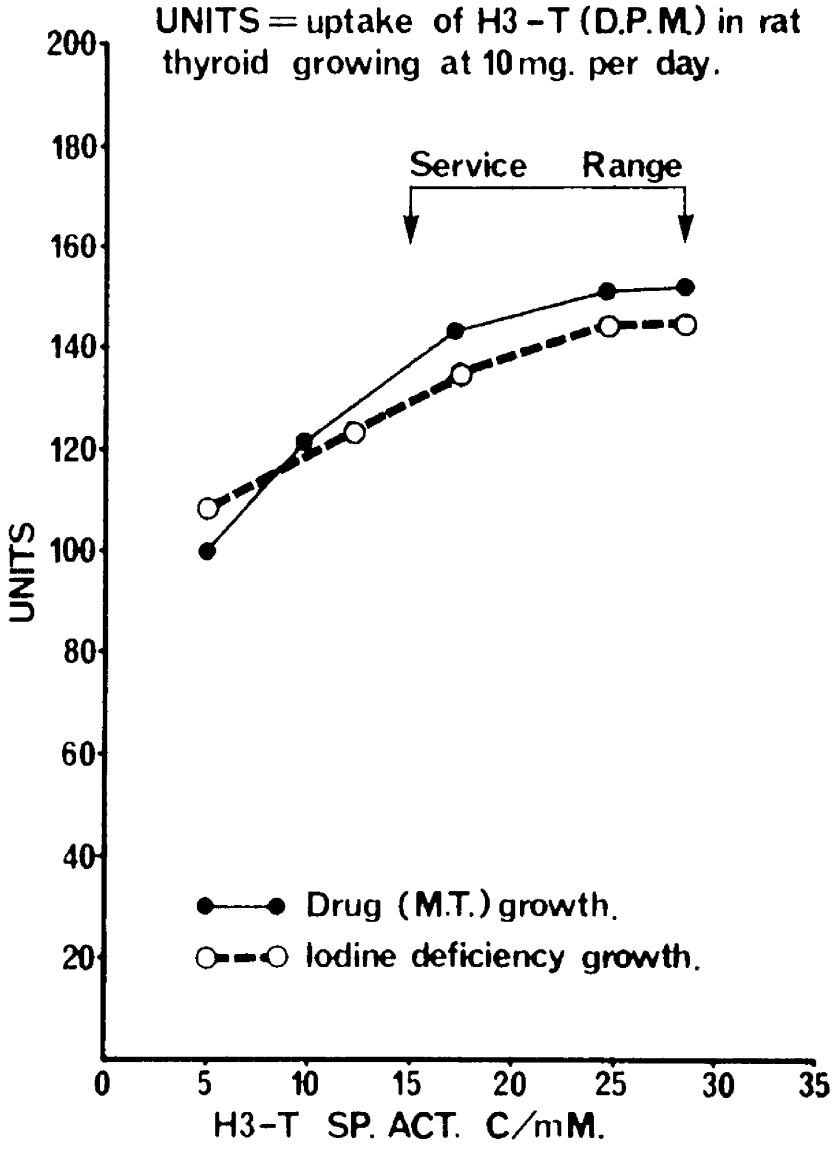


Fig. D-18 Rat thyroid cell D.N.A. tritiated thymidine (D.P.M.), 4 hours after single intraperitoneal dose (0.5 uci/gram). Effects of varying the SP. activity; iodine deficiency growth compared with methylthiouracil growth. Values are means from different pairs.

tritiated thymidine incorporation and labelling indices produced in the thyroid during the goitrogenic regime were not artefacts but reflected the real behaviour of the D.N.A. synthetic processes in normal cells undergoing proliferation. There is no reason to suspect that equal validation applied to X-irradiated thyroid but controls in all these above respects were not practical.

Discussion and Conclusions.

These studies demonstrate that unirradiated cells in normal adult rat thyroid synthesise D.N.A. and divide but at very low rates. The failure of a single dose of 500 rads of x-rays to perturb any of the parameters measured shows that any damage produced is not demonstrable using gland weight, cell density, chemical D.N.A. or tritiated thymidine incorporation as the indices (Fig. D-4). A single dose of 500 rads is a large dose by radiobiological standards, producing gross disturbances in cell populations in active proliferation (Alper 1967). Since there is no migration of cells to and from the thyroid and the thyroid cell population is mainly in G_0 or G_1 damage produced by 500 rads or more is latent. The other studies demonstrate this, since by promoting cell proliferation with a goitrogenic challenge marked changes are seen when non-irradiated and irradiated thyroid is used for comparison (Figs D-5, D-6, D-8, and D-11 to D-15 inclusive).

The effects of the goitrogenic challenge in non-irradiated animals appears mainly on nucleic acid synthesis, cell division, and organ growth, the rates of which, after a short lag, rise to

a maximum and decline quickly indicating that the system is self regulated (Figs. D-5 and D-6). The weight increase, due mostly to an increase in the number of cells, for example, increases quickly to approach asymptotically to a maximum weight. This restriction to a maximum weight appears to be due to a well regulated ceiling, the number of cell divisions being estimated as an average of not more than one or two per cell. The limitation of division appears to be intrinsic in the adult thyroid cells themselves a conclusion recently reached by Sheline (1969) too but the current studies, cannot show whether any of the cells have different intrinsic divisional capacities. The cell population has therefore to be considered as one population but with follicular or stromal cell subcompartments.

The irradiation effects (Figs. D-11 to D-15) brought out in thyroid subjected to goitrogen stimulation were x-ray dose dependent. However, only net thyroid growth as determined by weight at the end of a 28 day goitrogenic regime, co-related directly with x-ray dose (Fig. D-11).

In terms of the mode of action of x-rays the overall results are interpreted as showing that the principal effect of latent radiation damage in the thyroid is to reduce the proportion of cells which are able to divide when called upon to do so and the number of divisions they can complete but some effect on capillary integrity cannot be excluded (Stearner and Christian 1968). Although the rates of D.N.A. synthesis are

severely affected by x-rays (Figs. D-14 and D-15) they do not alone form a simple quantitative index of radiation damage. Continued D.N.A. synthesis without cell division and tissue growth makes interpretation of the radiation effect, if measured by D.N.A. synthesis alone, very difficult.

It would thus appear that the most appropriate simple index of crude cell survival after thyroid irradiation is the net impairment of thyroid growth to the whole 28 day goitrogenic procedure; but net weight is final thyroid weight minus initial thyroid weight. Weight increase not due to cell proliferation but to an increase in cell size or in the non-cellular structures in the thyroid must be considered before the system is adopted for quantitative cell survival studies. Cell hypertrophy is not a special feature of goitrogen stimulated rat thyroid since cells do enlarge and multiply in sequence and simultaneously (Johnson, 1969) so that there is a near constant relationship between weight, chemical D.N.A. and R.N.A. before and throughout the goitrogenic growth challenge (Fig. D-6). The relatively steady index of cell density in the same conditions (Fig. D-5) also suggests that increased cell number is the dominant change in goitrogen promoted thyroid growth. The small drop in cell density which does occur without or with irradiation (Fig. D-12) can be accounted for by a proportional loss of colloid, some small increase in average cell size and in the amount of collagen and vascular connective tissue (Santler 1957). We estimate that the fraction of net thyroid growth not due to cell division is about 10 per

cent. This is the order of the constant fraction of growth capacity retained after doses of X-irradiation of 1,200 rads and above (see Section E).

Thus when the rat thyroid, stimulated by a goitrogenic challenge, is to be employed as an index of residual thyroid cell survival not only must the goitrogenic regime be continued for at least 28 days but the growth should be expressed as net growth (final gland weight minus initial gland weight) and in addition a correction should be made for growth not due to cell division. The latter, about 10 per cent in value, should be obtained in each radiation experiment; this may be simply measured as the net growth which is constantly retained after doses of irradiation in the very high radiobiological range (e.g. more than a single x-ray dose of 1,200 rads).

In conclusion the rat thyroid may be employed as a model with which to study thyroid cell survival in vivo after irradiations. The special features of the model are that it is one of a highly differentiated tissue whose cells cannot divide indefinitely but perhaps only once or twice. It has, however, the advantage of relative simplicity and the results of experiments are relevant to the biological consequences and the therapeutic effects, of ionising irradiations on human thyroid. They are also likely to be relevant to clarifying radiobiological effects on organised differentiated tissues in general.

For example the studies are relevant to the observation

that irradiation and goitrogenesis are carcinogenic in rodents (Doniach 1963). The current studies explain why irradiation of the normal foetal, infant and child thyroid is much more likely to lead to subsequent hypothyroidism or thyroid cancer than irradiation of the adult organ, (Robbins, Rall and Conrad 1967, Wood, Tamagaki, Noriishi, Sato, Sheldon, Archer, Hamilton and Johnson 1969, Hempelman 1968, Dolphin 1968, Hollingsworth, Hamilton, Tamagaki and Beebe 1963). When the young thyroid, which has not completed its growth, is irradiated presumably further normal physiological growth is impaired and abnormal cells are left, the impairment of physiological growth being equivalent to the impairment of goitrogenic growth in the rat and studied above. Radiation impairment of thyroid cell reproductive integrity is also likely to explain why so many patients eventually become hypothyroid after iodine-131 therapy for thyrotoxicosis (Greig 1965) but this problem will be re-examined in detail in Section F. Finally, studies of the type described here may well allow investigations to prove or disprove some theories about the fundamental cell changes of radiation carcinogenesis (Mayneord 1968).

In the next Section (E) the effects of X-irradiation, iodine-131 irradiations and iodine-125 irradiations on rat thyroid cell proliferation and survival are compared. In the final Section (F) all the studies on gland structure (Section A), dosimetry (Section B), radiation effects on hormonogenesis (Section C) and radiation effects on cell proliferation (Section D) are collated with reference to the problems of radiation treatment of thyrotoxicosis.

SECTION E

COMPARATIVE EFFECTS OF EXTERNAL X-IRRADIATION, IODINE-131 AND IODINE-125 IRRADIATIONS ON RAT THYROID CELL SURVIVAL IN VIVO

Introduction.

As shown in Section D the rat thyroid is a suitable tissue for investigating the effects of ionising radiations on the proliferative capacity of thyroid cells *in vivo*. The normal rat thyroid, however, shows little change after irradiation, judged by weight, cell counts (total cells), cell proportions (follicular and stromal cells) or by changes in cell density or chemical D.N.A. and R.N.A. The apparent lack of effect of irradiation on the normal cell population arises, however, because the cells of the adult rat thyroid do not proliferate. This was formally demonstrated by negligible D.N.A. labelling indices both before and after high doses of external X-irradiation (Section D).

When, therefore, the effects of irradiations on rat thyroid cell proliferative capacity are to be measured *in vivo* it is necessary to promote maximum cell proliferation by giving the animals the goitrogen methylthiouracil in their drinking water. When this goitrogenic stimulus is given continuously for 28 days to non-irradiated rats (control) it was found that the thyroid weight increased 2 to 3 fold, the cell numbers increased and the proportion of stromal cells rose from about 30 to 42 per cent; in addition total gland chemical D.N.A. and R.N.A., and the nuclear incorporation of tritiated thymidine (total incorporation and cell labelling indices) rose (Section D). It would appear that all but about 10 per cent of the mean net weight increase (mean weight after goitrogen minus mean weight before goitrogen) is specifically due to follicular and stromal cell proliferation. Evidence that

some weight increase is not due to cell division is also seen in the retention of residual growth after very high doses of x-rays. Nevertheless if rat thyroid is first irradiated and then the animals are put on the goitrogen, thyroid weight increase is impaired and the degree of impairment is clearly related to x-ray dose. While most of the effects are due to interruption of follicular and stromal cell proliferation, the small proportion of net thyroid weight increase which is not due to cell proliferation must be subtracted before the final results may be expressed as cell survival. The above procedure has been used in this section (E) in which in vivo radiation dose survival graphs from X-irradiated and iodine-131 or iodine-125 irradiated rat thyroid cells are compared.

The X-irradiation was given as single doses, the dose-rate being high and steady and the dose distribution homogeneous, at every dimension within the rat thyroid (Section B). For these reasons the X-ray survival data was used as the standard reference with which to compare the effects of iodine-131 or iodine-125 irradiations neither of which gave high nor steady dose rates and both of which irradiate the rat thyroid inhomogeneously. There are, however, as shown in Section B important differences between the inhomogeneity of dose from iodine-131 and that from iodine-125 respectively. Both isotopes are located within the colloid during their stay in the thyroid (Sections A and B) and the irradiations emitted by each radiate the surrounding follicular cells (Fig. E-1). Whereas iodine-131 irradiates the central parts of the gland more

DOSE DISTRIBUTION (I-131 and I-125) IN RAT THYROID.

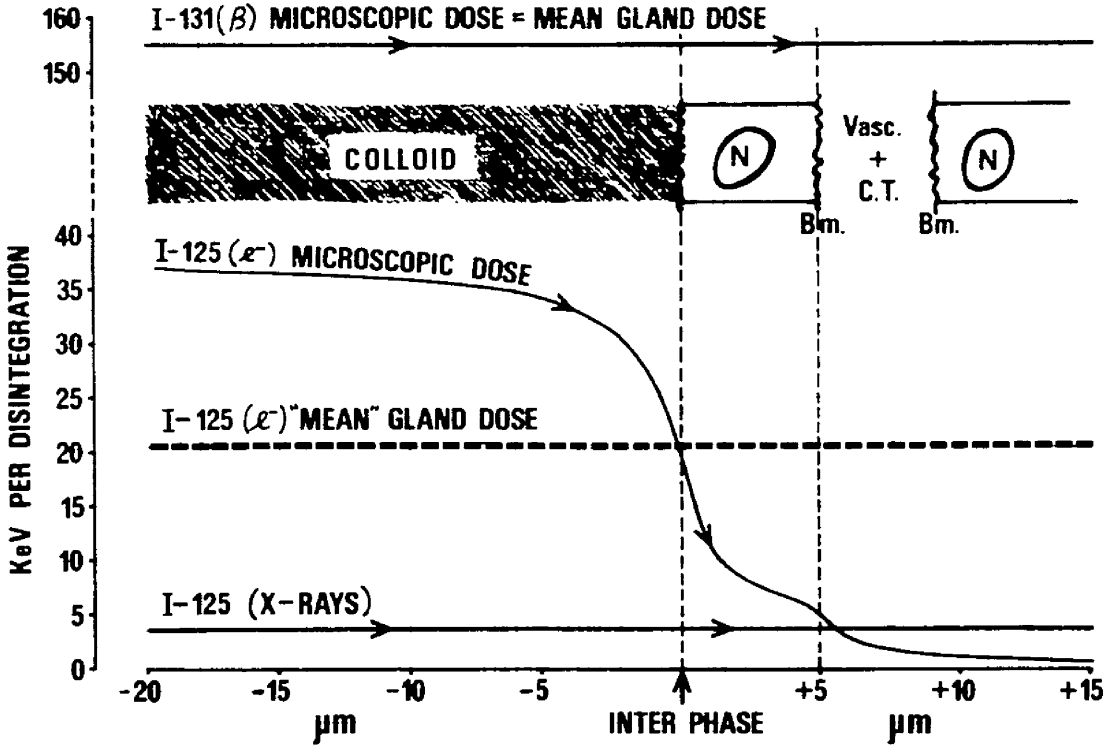


Fig. E-1 This is also Fig. B-6. Calculated dose (KeV) dose distribution in a segment of rat follicle arising from equal concentrations of iodine-131 or iodine-125 (90 per cent in colloid). $\mathscr{e}$ is electron dose, N is nucleus, Bm is basement membrane, Vasc. + C.T. is vascular plus connective tissue.

than a very thin outer rim of about 15 per cent of total volume the cytoplasm and nuclei of the cells are irradiated with equal intensity; in contrast the radiation doses from iodine-125 are twice as high at the colloid end of the follicular cells (apices) than at their basal ends (nuclei). As a consequence iodine-125 irradiation doses inside the bodies of the follicular cells are not homogeneous, the cell nuclei receiving relatively 50 per cent of the mean dose to the gland. These special dosimetric aspects prompted an investigation of the relative cell survivals after x-rays, or iodine-131 or iodine-125.

The experiments were conducted in three phases, an irradiation phase, a lag phase, and a phase of goitrogen administration lasting 28 days. At the end of this phase the rats were killed, thyroid weights determined and finally the data were adjusted to calculate thyroid cell survival. 15 rats were not given irradiation nor goitrogen to obtain normal thyroid weight.

Irradiation Procedures and Dosimetry.

Not less than 15 rats per dose group were given external X-irradiation to the neck, but not to the pituitary, in single doses up to 1,800 rads as described in Sections B and D. The dose-rate to the thyroid in this experiment was 570 rads per minute. Not less than 15 rats per dose group were given iodine-131 or iodine-125 as single intraperitoneal injections in 1 or 1.25 ml of normal saline. The amounts injected varied from 2.5 to 40 uCi of iodine-131 and from 2.5 to 320 uCi of iodine-125. All animals

were kept under standard conditions, normal diet and water for six weeks after the injection of radioactive iodine. During and at the end of this interval sub groups given 10 uCi of iodine-131 or 40 uCi of iodine-125 were killed to obtain data for dosimetric calculations as described in Section B. For dosimetric calculations a total of 85 rats were killed at intervals up to 6 weeks after the injection of radioactive iodines and data on mean thyroid mass, mean maximum thyroid uptake and mean thyroid biological half-life of radioiodine were obtained.

Measurement of Cell Survival.

One month after X-irradiation or 6 weeks after the administration of iodine-131 or iodine-125, all rats, irradiated and non-irradiated were given the goitrogen for 28 days as described in Section D. At the end of this phase they were killed with coal gas and each thyroid was resected and weighed to the nearest 0.1 mg. Since there were not less than 15 rats per radiation treatment group the mean thyroid weights were used for calculation of cell survival. This was done as follows. First the mean net weight increase was determined by subtracting the mean weight before the goitrogen (normal) from the mean weight after the goitrogen for each radiation (and non-radiation)group. These data were then converted to percentages, the non-irradiated mean thyroid weight increment (control) being 100 per cent. This information, representing net thyroid growth (per cent), was then formally plotted against each radiation dose. The percentage thyroid growths persisting after the very highest doses of each

irradiation were thus determined. These percentages were considered to represent the proportion of thyroid growth not due to cell proliferation (see above) and were employed as factors with which to return to and adjust the raw gland weight data to represent cell survival. For example, after the highest doses of X-irradiation there was a 10 per cent residual thyroid growth and so the mean thyroid weight increment after each x-ray dose (including 0 rads) was reduced by 10 per cent and when the data were recalculated as a percentage, with adjusted non-irradiated thyroid weight increment as 100 per cent, the information was named thyroid cell survival.

Results.

Mean Radiation Doses - Iodine-131 and Iodine-125.

The mean weight of 85 rat thyroids taken sequentially over 6 weeks for dosimetric calculations was 22.1 mg and the mean maximum thyroid uptake was 20.5 per cent of the dose administered. This maximum uptake occurred at between 18 and 24 hours after the isotope was injected. The mean biological half-life of the thyroid radioiodine was 8 days so that the effective half-life of iodine-131 irradiation was 4 days (physical half-life = 8 days) and that of iodine-125 irradiation was 7.1 days (physical half-life = 60 days). Because the maximum uptake was reached comparatively very much quicker (18 - 24 hours) than the effective half-life (4 days and 7.1 days respectively) the radiation doses delivered during the uptake phase was considered negligible (Loevinger, Holt and Hine 1958). The mean β or electron doses throughout

TABLE E-1

Percentage Weight Increase and Calculated Percentage Cell Survival after X-irradiation, and Iodine-131 or Iodine-125 Irradiation of Rat Thyroid (values are means)

X-rays			Iodine-131		
Dose Rad	Percentage Thyroid Weight Increase	Percentage Cell Survival	Dose Rad (uCi given)	Percentage Thyroid Weight Increase	Percentage Cell Survival
0	100	100	0 (0)	100	100
100	97.7	97.0	530 (1.25)	100	100
200	93.3	93.0	1,060 (2.50)	95.5	95.0
400	69.2	65.5	2,120 (5.00)	79.4	77.0
500	53.7	48.0	4,240 (10.0)	44.7	39.0
800	37.2	30.0	6,360 (15.0)	37.2	30.0
1,000	24.5	17.0	12,700 (30.0)	20.9	12.0
1,200	19.1	11.0	17,000 (40)	15.2	5.7
1,500	12.6	4.0	25,500 (60)	11.2	1.3
1,800	11.4	2.7	50,900 (120)	11.4	1.5

TABLE E-1

Iodine-125		
Bone Rad (rad given)	Percentage Thyroid Height Increase	Percentage Cell Survival
0 (0)	100	100
550 (5.5)	100.6	100
550 (5)	102.2	100
1,170 (10)	99.4	98.0
2,330 (20)	91.3	92.5
3,500 (30)	87.1	86.0
4,670 (40)	70.9	67.0
7,000 (60)	56.2	50.5
9,330 (80)	45.2	37.0
11,700 (100)	40.7	32.0
14,000 (120)	35.5	26.0
16,300 (140)	26.3	15.2
18,700 (160)	24.6	13.3
23,000 (180)	22.4	10.0
25,700 (220)	19.5	7.3
32,700 (280)	17.0	4.6
42,000 (360)	14.3	1.5

the gland were therefore calculated exactly as described in Section B ignoring the γ ray dose from iodine-131 but adjusting for a 20 per cent loss of β-radiation from the surface and a 15 per cent shortening of B.H.L. at high doses (30 uci or more iodine-131). The x-ray dose from iodine-125 (Fig. E-1) was included and no adjustment was necessary for surface loss of electron dose. A correction for 15 per cent shortening of B.H.L. was made after iodine-125 doses of 120 uci or more. The reasons behind the above are given in Section B.

In these experiments the calculated mean doses to the rat thyroid were as follows. The mean thyroid dose from iodine-131 was 424 rads per uci administered and this was the mean dose to the follicle cell cytoplasm and nuclei. The mean thyroid dose from iodine-125 was 117 rads per uci administered and this was the mean dose at the follicle cell apex too. The mean dose to the follicle cell nuclei was, however, about half this (see Fig. E-1).

Thyroid Cell Survival after X-rays, Iodine-131 or Iodine-125.

The survival data will be presented using mean gland doses, but the radiobiological significance of the characteristics of the survival graphs will be interpreted in the light of the differences between the three irradiations with respect to dose rates, quality and especially cell distribution of doses and mean nuclear doses.

Table E-1 is a summary of the experiments and the results. In the table "percentage thyroid weight increase" refers to the mean net weight increase per group (minimum of 15 animals per group)

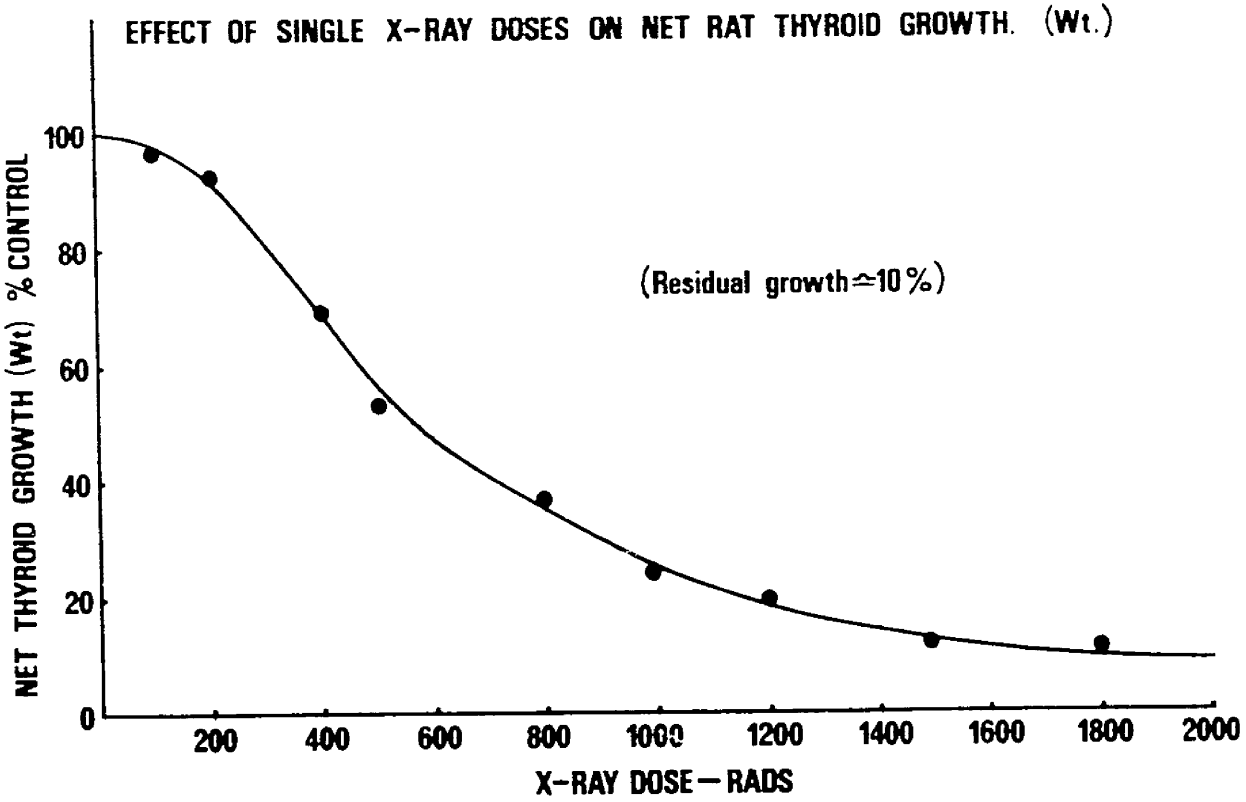


Fig. E-2 Net rat thyroid growth (wt.) induced by 28 day goitrogenic regime after X-irradiation. (See text)

after the 28 day goitrogenic regimen, a percentage of 100 being that of non-irradiated controls subjected to the same goitrogenic procedure. With increasing radiation doses there was progressively less thyroid weight increase but even after doses of X-irradiation as high as 1800 rads there was a residual thyroid growth of 11.4 per cent. Complementary values for residual thyroid growth after the largest doses of iodine-131 and iodine-125 irradiations were 11.4 per cent and 14.3 per cent respectively.

"Percentage thyroid weight increase" was, however, formally plotted against mean radiation dose for each radiation and the plots were extended to the "plateau" regions. As an example the plot of thyroid growth per cent against the x-ray dose is shown in Fig. E-2. With x-rays the "plateau" was approximately 10 per cent. The "plateau" for iodine-131 irradiation was also approximately 10 per cent and that for iodine-125 irradiation was 13 per cent. As discussed above these values were taken to represent residual thyroid weight increase, not due to cell proliferation, and therefore to calculate cell survival they were used as adjustment factors as described above. Cell survival are shown in Table E-1 as the column "percentage cell survival". These data also are shown on a logaritchmic scale in Fig. E-3. Table E-1 also has columns for mean gland absorbed doses from x-rays iodine-131 and iodine-125 and these data are likewise shown in Fig. E-3.

Fig. E-3 thus shows that the x-ray cell survival curve

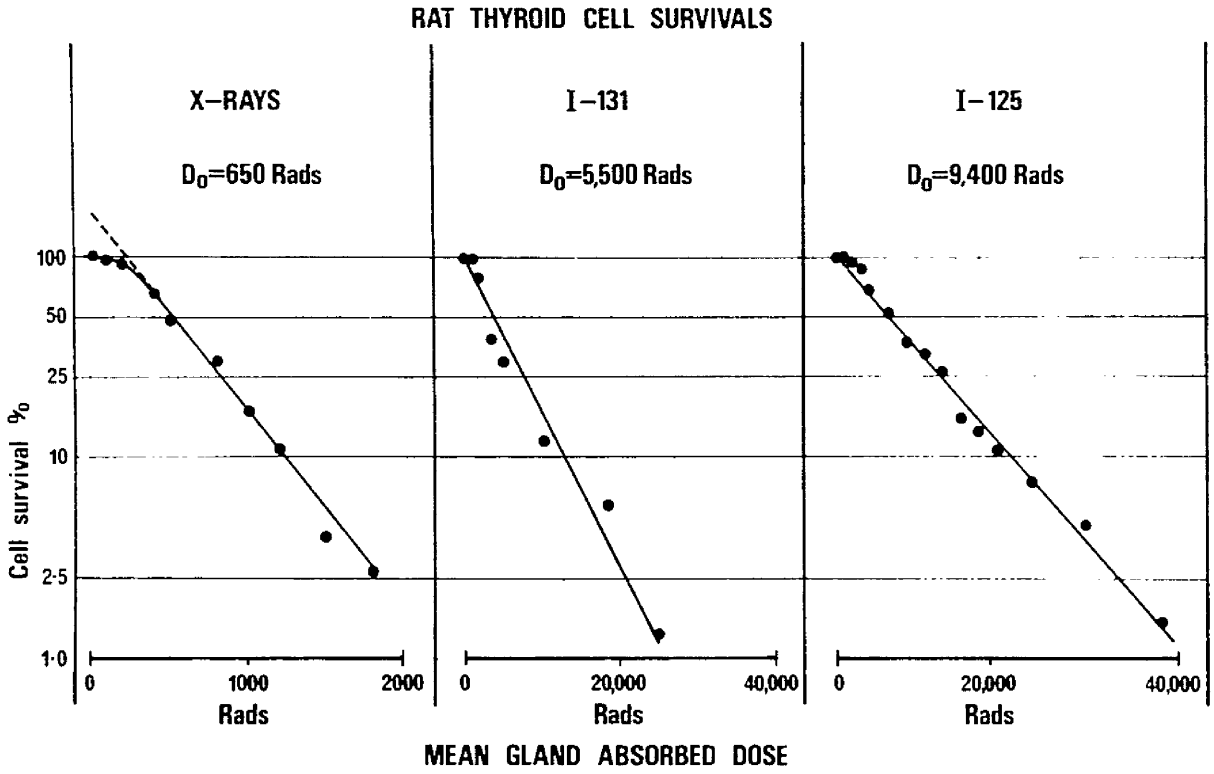


Fig. E-3 Mean percentage thyroid cell survival (adjusted for non-divisional growth) resulting from rat thyroid irradiation by x-rays, iodine-131 and iodine-125 and tested for with a 28-day goitrogenic challenge. D_o values refer to mean gland dose only.

exhibits an initial shoulder followed by an exponential decrease; the exponential part has a D_o of 650 rads and an extrapolation number of 1.7. The iodine-131 cell survival data lie in an exponential line passing through the origin without a shoulder; the D_o is 5,500 rads. The iodine-125 cell survival points also lie in an exponential line through the origin although there is a suggestion of a very slight shoulder. The D_o is 9,400 rads. The D_o values shown in Fig. E-3 refer to mean gland radiation doses without reference to dose-rate, quality or to non-homogeneity of dose and they are in respect of a 28 day goitrogen produced cell proliferation in normal but irradiated rat thyroid (i.e. irradiation of thyroid cells in ~~situ~~ vivo).

Interpretation and Discussion.

In the absence of irradiation a 28 day goitrogenic regime results in a 2 or 3 fold increase in the weight of the thyroid and this increase is largely due to cell proliferation (Section D). Irradiation given before the goitrogenic stimulation results in a smaller weight increase and this impairment appears to be the consequence of a specific effect of the irradiation on the proliferative capacity of the thyroid cell population (Section D) and as discussed by Gibson and Doniach (1967), Al-Hindawi and Wilson (1965), Dobyns, Rudd and Sanders (1967).

In the present investigation the effects of prior irradiation with single doses of x-rays or iodine-131 or iodine-125 on goitrogenic rat thyroid cell proliferation were compared. The effects, in terms of specific cell survival, could only be

measured after account was taken of the component of thyroid growth considered not to be due to cell division (Fig. E-2). Comment on the characteristics of the survival data (Fig. E-3) required in addition that the special characteristics of cell multiplication in the thyroid discussed in Section D were evaluated.

Only about 85 to 90 per cent of the increase in the weight of the thyroid produced by goitrogenic stimulation, in the absence of irradiation, is due to cellular division (Philp et. al. 1969, Chow and Woodbury 1965, Matovinovic and Vickery 1959, Lindsay and Cohen 1965, Harkness, Harkness and Santler 1954), the residual growth being cell hypertrophy or non-cellular. This probably explains why very large doses of the irradiations failed to abolish all goitrogen induced thyroid growth (Table E-1). This is shown formally in Fig. E-2, for example, where the thyroid growth, plotted against x-ray dose, shows residual growth of about 10 per cent (x-rays irradiate the thyroid uniformly so that there are no parts in which cells are spared). Residual rat thyroid post-radiation growth was noted in previous studies of this type (Abbatt, Doniach, Howard-Flanders and Logothetopoulos 1957, and Greig, Crooks and MacGregor 1966) and in other tissues such as the irradiated mouse testis (Kohn and Kallman 1954). The proportion of residual growth for the three types of irradiation were almost equal, being about 10 per cent in the x-ray experiment, about 10 per cent in the iodine-131 experiment and about 13 per cent in the iodine-125 experiment. It thus appeared necessary to

subtract the component of weight increase not abolished by irradiation before the term cell survival could be applied to the data. While it is appreciated that this method is somewhat empirical a more objective method is not known to the author.

Before the features (shoulder and D_o) of the three radiation cell survival plots (Fig. E-3) can be fully interpreted however, it is also necessary to briefly consider the special characteristics of rat thyroid cell proliferation induced by a goitrogen over 28 days. The characteristics of the thyroid cell proliferation which was impaired in these studies is different from the almost unlimited cell multiplication potential observed in classical cell survival studies using clonogenic systems (Puck and Marcus 1956, Elkind 1967, Whitmore, Gulyas and Botond 1965, Lamerton 1968). Normal unirradiated follicular and stromal thyroid cells <u>in vivo</u> can certainly be induced to undergo an average of one or two divisions by the goitrogenic challenge but the maximum number of divisions is probably not more than four (Section D and Sheline 1969). This means that even unirradiated thyroid cells <u>in vivo</u> could not be regarded as reproductive in clonogenic terms; since follicular and stromal cells are capable of only a few divisions without irradiation, cell irradiation must stop the earliest divisions before an effect on organ growth is demonstrable. In other words, the thyroid studies described here represent impairment of the <u>first or second post-radiation divisions</u>. In radiobiological studies with clonogenic systems,

cells which can only achieve the first few divisions are not counted as survivors since small clones are often ignored. Since larger doses of irradiation are required to abolish the immediate post-radiation divisions than the later divisions (Nias 1968), the D_o of systems like the rat thyroid would therefore be expected to be higher than those found in classical clonogenic systems (Fowler et al. 1963 and Lamerton 1968). Hence perhaps it is not surprising that the D_o of the x-ray curve was 650 rads (Fig. E-3). It is not therefore necessary, in my opinion, to postulate an unusually radio-resistant population or hypoxic conditions in thyroid cells in vivo to explain this high D_o value.

It is now possible, using the x-ray data in Fig. E-3 for reference, to consider and compare the effects of iodine-131 and iodine-125 irradiations on cell survival. The iodine-131 and iodine-125 irradiations in the thyroid differ from those of the X-irradiation in three chief respects; the dose rates are different, the qualities are different and the dose distributions are different. It is expedient first to compare the x-ray effects with the iodine-131 effects since both irradiated the rat thyroid cells homogeneously although a very narrow rind of gland receives a lower dose than average from iodine-131 (Section B). The β energies from iodine-131 are, however, high enough to have an R.B.E. close to unity and to that of x-rays in respect of cell reproductive integrity (Bacq and Alexander 1955). Therefore the only relevant differences between x-rays and iodine-131 were the dose rates.

The x-ray and iodine-131 curves (Fig. E-3) differ in D_o

and in the shoulder, the iodine-131 having a much higher D_o (5,500 rads) and no shoulder. Both these features of the iodine-131 graph would be consistent with the effects resulting from lowering of the dose-rate. The elimination of the shoulder by an extension of the times in which the iodine-131 doses were given implies that the shoulder represents the repair of sublethal damage to divisional potential. Since the repair process had time to operate continuously with low dose rates from iodine-131 more absorbed dose is required to give equivalent effects and hence the D_o must rise. Attempts have been made in other circumstances to quantify and to predict the sparing effects of low dose rates (Elkind 1967). The basis of these predictions was usually by extrapolation from high steady dose-rate survival curves and split dose recovery curves (Elkind 1967 and Lamberton 1968). In the current problem, however, not only were there no specific data on the recovery phenomenon but the dose rates arising from the iodine-131 irradiation were not simple; although average rate was proportional to total radiation dose the dose rates actually fell exponentially. It is, however, relevant that although the maximum dose rates at peak iodine-131 thyroid uptake (18 - 24 hours) varied from 10 to 400 rads per hour (calculated) corresponding to the increasing amounts of activity (uCi) administered the survival curve remained exponential (Fig. E-3). This finding itself suggests that variations in dose-rate within this range do not affect the quantity of repair of sublethal damage to reproductive integrity in the rat thyroid cells.

It is concluded therefore that the D_o value of 5,500 rads arising from iodine-131 irradiations in rat thyroid is due to a combination of a slow average dose-rate and the special divisional limitations of the tissue cells. It is unlikely that the radiation quality (R.B.E. = 1) or slightly inhomogeneous distribution of dose contributed to the high D_o value by sparing tissue. When, however, the features of the iodine-125 survival data (Fig. E-3) are now considered and compared with those of x-rays and iodine-131 respectively, the factors of radiation quality and inhomogeneous dose at the cell level in addition to that of dose-rate must be discussed.

Comparison of the iodine-125 and iodine-131 survival curves for mean gland doses (Fig. E-3) shows that the D_o is much higher after iodine-125 irradiation (9,400 rads) than after iodine-131 irradiation (5,500 rads) an R.B.E. difference of 1.7. Although the effective half-life (E.H.L.) of the iodine-125 was somewhat longer (7.1 days) than that of iodine-131 (4.0 days) higher mean gland iodine-125 absorbed radiation doses were required to produce survivals equivalent to those of iodine-131 (Table E-1 and Fig. E-3). Since average dose rates were related to total doses and to durations (E.H.L.) it was found by calculation that, for equivalent cell effects, the average dose rates were not different by more than 10 per cent when iodine-125 was compared to iodine-131 irradiation. Thus the high D_o of iodine-125 could not be explained on the basis of a much lower dose-rate.

The other possibility that the R.B.E. of the iodine-125

irradiations on cellular reproduction was less than 1.0 was considered. However, the predicted R.B.E. of iodine-125 irradiations is in general greater than 1.0 (Fiege and Gross 1968).

The remaining possibility that the inhomogeneous distribution of radiation dose which results in a dose to follicular cell nuclei and to stromal cells about half the mean dose was therefore thought to be the chief reason why the D_o of iodine-125 was so high when calculated on the basis of mean gland dose only. In reality the mean dose to cell nuclei was at least 50 per cent of that to the gland as a whole (Fig. E-1). Thus if recalculated as the mean dose to cell nuclei the iodine-125 D_o would be much nearer the D_o value for iodine-131 (5,500 rads).

All these studies therefore tend to show that it is the mean dose to follicular cell nuclei which determines injury to reproductive integrity or cell survival and this applies whether the cell cytoplasm is homogeneously irradiated or not. With x-rays and iodine-131 follicular cell cytoplasm and nuclei received the same doses but with iodine-125 the inner aspect of the follicular cells received more irradiation than nuclei; nevertheless cell survival appears to be determined by mean doses to nuclei.

With iodine-125 the stromal cells which comprise about 30 per cent of the total cell population are relatively spared compared to iodine-131 or X-irradiation (Fig. E-1) and a small part of the relatively increased total cell survival after iodine-125 may be due to some sparing of the stromal cell population. Indirect evidence for this was obtained in a subsidiary experiment,

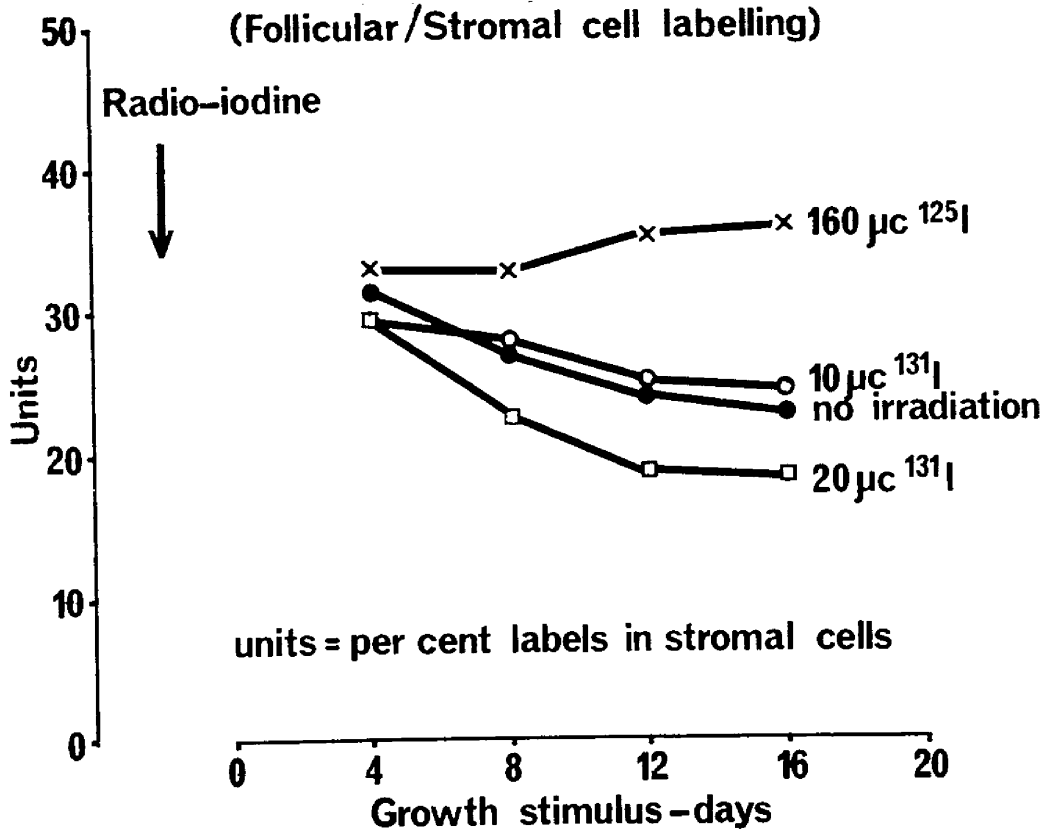


Fig. E-4. Tritiated thymidine labelling of stromal cells proliferating units = per cent of all labelled cells (50 fields) which were stromal cells. Iodine-125 irradiation allows more stromal cell proliferation.

in which groups of 5 rats given different doses of iodine-131 and iodine-125 and subjected to the goitrogenic challenge had their thyroid follicular and stromal cells, in S phase, labelled with a pulse of tritiated thymidine as described in Section D. Selected data from this experiment are shown in Fig. E-4 which illustrates the percentage of all labelled cells (in 50 fields) which were labelled stromal cells during thyroid growth. Fig. E-4 demonstrates that the proportion of cells in proliferation which are stromal cells was relatively greater after iodine-125 than after iodine-131 or in the absence of irradiation (no irradiation).

In addition to holding general radiobiological and radiotherapeutic interest these studies are relevant to the problems of radioactive iodine therapy for thyrotoxicosis. When iodine-131 is used to suppress excessive hormonogenesis of the thyrotoxic thyroid cell population, the irradiation doses to the follicular cell nuclei are the same as those to the parts of the follicular cells which complete hormone synthesis. Furthermore, these doses are more or less equal throughout the thyroid. As a result the high doses required to impair hormonogenesis because it is radioresistant (Section C) create severe nuclear injury because it is relatively radiosensitive (Sections D and E) and the follicular cell populations fail to survive in sufficient numbers to maintain cell renewal and prevent a rising incidence of hypothyroidism. The high iodine-131 irradiation doses given to the interfollicular stroma probably contribute to the organ failure as postulated by

Greig (1965). However, iodine-125 delivers higher doses to some of the hormonogenetic parts of the follicular cells than to the cell nuclei or interfollicular stroma (Section B). Thus thyrotoxicosis might be controlled with iodine-125 therapy without subsequent cell failure and hypothyroidism. These studies are described in the next section together with a short review of the pathogenesis and general management of thyrotoxicosis (Section F).

SECTION F

RADIATION EFFECTS ON THYROID HORMONOGENESIS AND CELL PROLIFERATION IN RELATION TO TREATMENT OF THYROTOXICOSIS.

Introduction.

Thyrotoxicosis is a common and potentially lethal disorder which, as far as is known affects only humans; it is characterised by spontaneous excessive and sustained secretion of thyroxine (T4) and tri-iodothyronine (T3), and about 5000 patients per 50 million population in Western Europe, Scandinavia, Great Britian, the U.S.A. and Canada present for treatment each year. The clinical disorder is very much more prevalent in women and about 60 per cent of all patients are over 40 years of age. Although clinical thyrotoxicosis has been recognised to be a distinct thyroid disorder for decades it is only in the last 20 years that accurate laboratory measurements, which distinguish this order from other disorders of the thyroid gland, have been available and only very recently has some meaningful data about some of the primary pathogenic mechanisms been provided.

The aim of this final section (F) is to briefly review current ideas about the pathogenesis of thyrotoxicosis and modern treatment. Particular emphasis has, of course, to be placed in the present account on the therapeutic uses of ionising irradiations.

Pathogenesis, Structure, Hormonogenesis and Cell Proliferation in Thyrotoxic Thyroid.

Pathogenesis.

Many patients with thyrotoxicosis have diffuse enlargement of the thyroid. The enlargement varies very greatly however in degree and symmetry; the gland may weigh 30 grams or 300 grams but

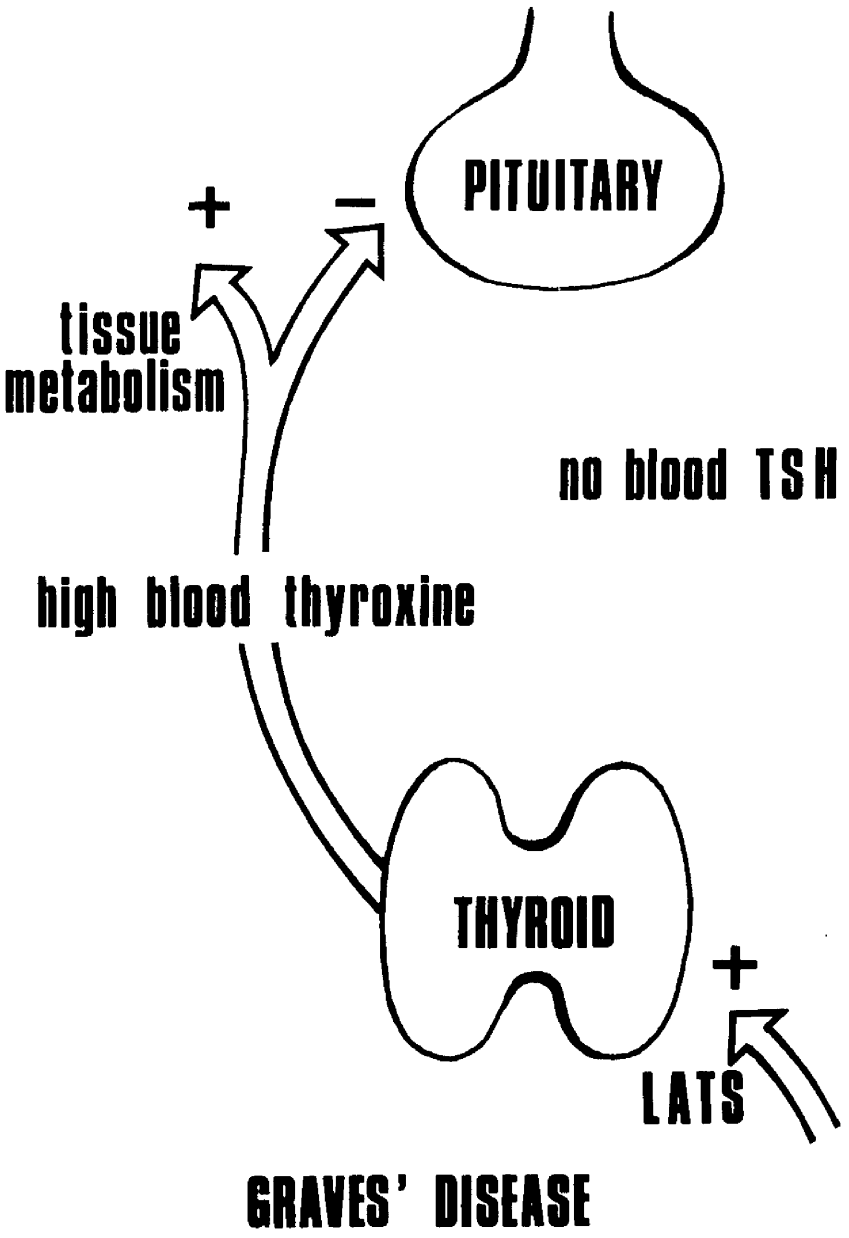


Fig. F-1 From Adams (1965). L.A.T.S. arises outside pituitary and stimulates the thyroid to produce excessive thyroxine.

most patients have a goitre weight of between 40 and 60 grams. Often one lobe is larger than the other and nodular formation of varying degree (size) and extent (number) is quite common. Only a very small number of thyrotoxic patients (about 5 per cent) have a single discrete autonomously hyperfunctional adenoma in an otherwise normal thyroid. This small group of exceptional patients will not be further considered here because their thyrotoxicosis is not due to the mechanism(s) which cause the disease associated with general thyroid enlargement. Patients with a toxic adenoma are best treated by surgery since removal of adenoma cures the thyrotoxicosis.

The common type of thyrotoxicosis (more than 90 per cent of patients) with general enlargement of the thyroid (Grave's Disease) arises because the patients have in their blood an immunoglobulin (7S, IgG globulin) which has the specific and unique effect of stimulating mammalian thyroid follicular cells to form and secrete excessive amounts of T4 and T3. This substance called the long acting thyroid stimulator (LATS) because of its capacity to cause more prolonged secretion of thyroid hormone than T.S.H. in animals (Adams 1965) and man apparently arises from unknown sites in human lymphoid tissue (Fig. F-1). Whether, in predisposed individuals, LATS is spontaneously produced in levels high enough to bring about clinical thyrotoxicosis or whether some adjuvant stimulus plays a part is not yet known. It would, however, appear that provided the blood titre of LATS is high and sustained and the thyroid gland is intact hormone secretion rises; as far as can be determined the

gland is not initially, structurally or hormonogenetically abnormal. It would seem that all the morphological and functional changes are brought about by the action of L.A.T.S. These changes are indistinguishable in every respect from those which can be observed *in vitro* or *in vivo* when human or rodent thyroid is subjected to exogenous T.S.H. stimulation (Adams 1965, McKenzie 1967, 1968, Kriss 1968, Burke 1968, Metzel 1968, Ochi and De Groot 1968).

Structure.

At the microscopic level there is a relative lack of quantitative data on the structure of the untreated thyrotoxic gland since tissue from untreated patients is now rarely available. Studies with conventional light microscopy carried out before effective treatments were available (Wilson 1927) can, however, be complemented with recent electron-micrographic data on biopsy tissue and a reasonable assessment of the microanatomy of the typical untreated human thyrotoxic gland can thus be made (Heimann 1966).

In the thyrotoxic thyroid the characteristic light microscopy changes are increased vascularity, increased absolute and relative numbers of follicular cells and decreased amounts of colloid thyroglobulin. In a typical follicle, the orderly spherical arrangement of a smooth ring of flat or cuboidal follicular cells and a spherical colloid core in normal thyroid is replaced by marked infolding and convulution of the follicular cell boundary; in addition the individual follicular cells are greatly elongated

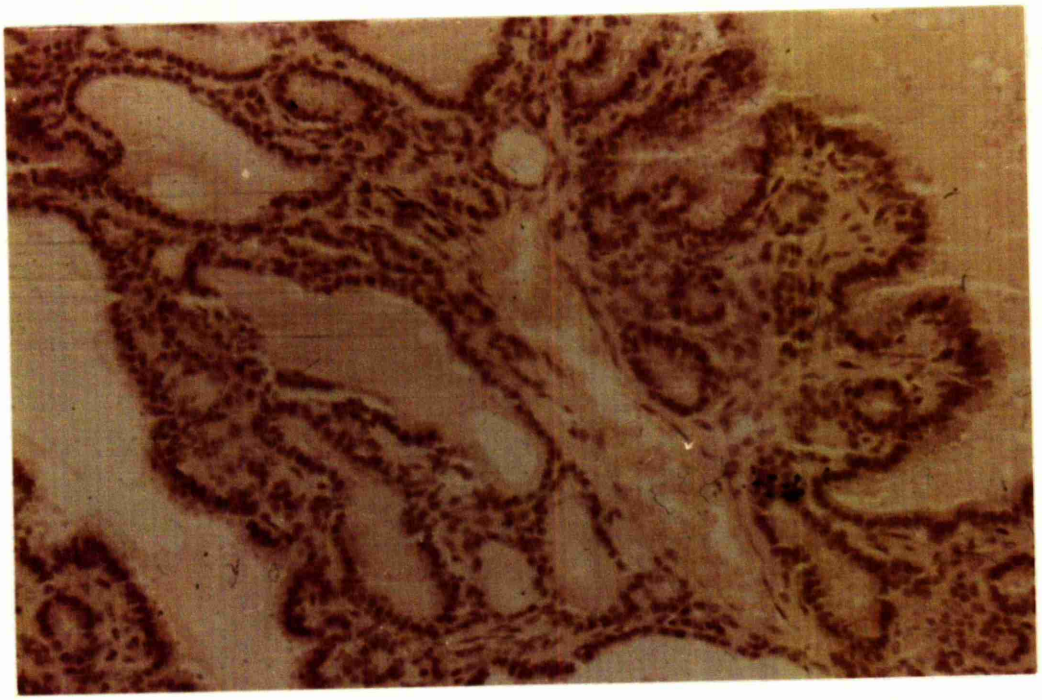


Fig. F-2 Light microscopy of untreated thyrotoxic thyroid. Note vascularity, irregular colloid lumen and elongated follicular cells.

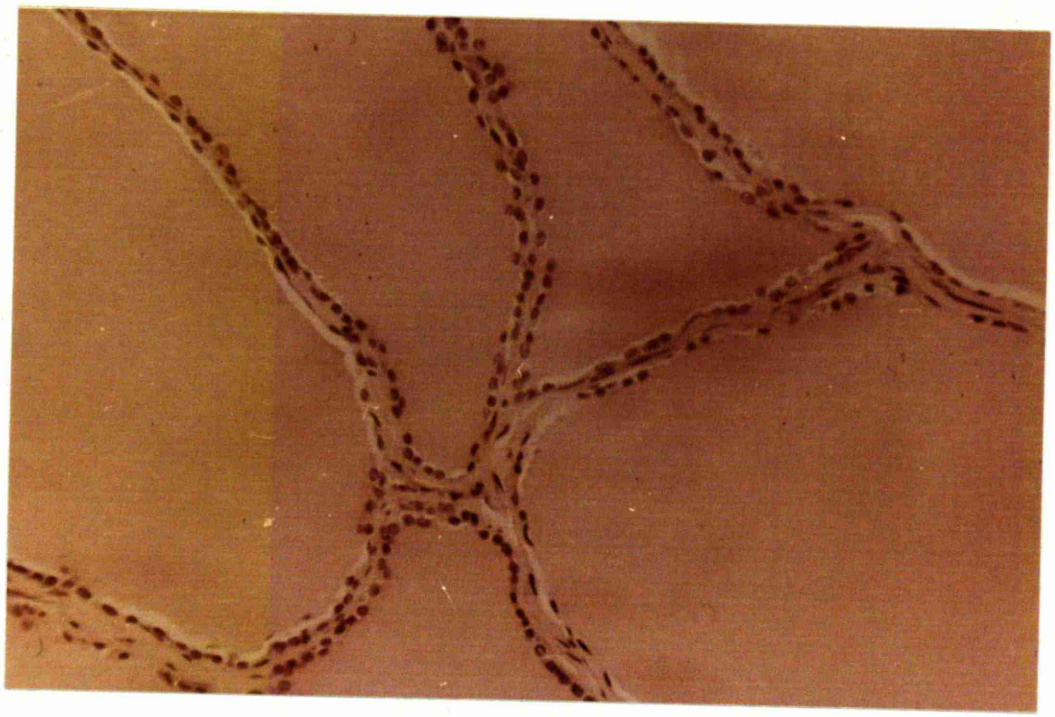


Fig. F-3 Light microscopy of normal adult human thyroid.

and columnar. These changes have the effect of creating an undulating pallisade of columnar follicular cells involuting an irregular narrow follicle lumen of colloid (Fig. F-2 and F-3); these changes are brought about by the action of LATS and are adaptations which accompany the increased rate and quantity of hormonogenesis.

Only a small number of the follicles are spherical and they have diameters of between 20 um and 150 um. Most of the follicle lumens are irregular and slit-like. In the thyrotoxic thyroid the colloid volume may be decreased to as little as 5 - 10 per cent of the total gland volume and is probably seldom more than 30 per cent of it. The average proportion of total gland volume due to colloid appears to be about 20 per cent (Heimann 1966). A much greater proportion of the volume is however due to vascular space (20 per cent) and follicular cells (60 per cent). Of all the cells in an average thyrotoxic thyroid follicular cells probably comprise 80 per cent, 9 per cent being stromal cells and 1 per cent parafollicular cells (personal estimate).

As stated the follicular cells are typically elongated and columnar. The lengths vary between 10 um and 30 um with an average of approximately 15 um. Their width remains about 5 um but the nuclei (4 x 3 x 3 um) lie typically at the basal ends of the follicular cells at least 5 - 10 um away from the colloid apical boundary (Fig. F-2).

Hormonogenesis.

Electron micrographs of untreated thyrotoxic thyroid show all

Fig. F-4 Electron micrograph of untreated thyrotoxic thyroid. Follicular cell microvilli are numerous and long (x 51,000). Arrow indicates pinocytosis.

From Heimann 1966.

Fig. F-5 Electron Micrograph of untreated thyrotoxic thyroid. Large follicular cell apical cytoplasmic projections containing vesicles, ribosomes and cisternae (ci) of endoplasmic reticulum (x 22.000).

From Heimann 1966.

changes consistent with L.A.T.S. (T.S.H. like) stimulation of rates of hormone formation, colloid resorption, thyroglobulin proteolysis and hormone secretion. These ultramicroscopic changes are an increased number and volume of mitochondria, ribosomes, endoplasmic reticula and a large Golgi organ. The apical membrane has a much greater number of microvilli than normal and these are longer than normal (Fig. F-4). The number of lysosome bodies and intracellular colloid droplets are also increased and projections of apical endoplasm are seen in colloid margin (Fig. F-5). Although a great amount of work has been done with the object of defining a single critical biochemical action and site of action of L.A.T.S. (and of T.S.H.) none have been demonstrated; one possible site is the cell apical membrane (Burke 1969). In respect of all biochemical and morphological changes induced in mammalian thyroids L.A.T.S. and T.S.H. appear to be indistinguishable. There is some evidence, however, to indicate that in thyrotoxicosis the thyroid is intrinsically normal, that all aspects of hormonogenesis although accelerated and increased quantitatively are qualitatively not overtly abnormal. For example, thyrotoxic thyroid tested in vitro produces 19S thyroglobulin (Thomson and Bissett 1969). As in the normal rat, all thyrotoxic follicular cells appear to both form and store thyroid hormones in the thyroglobulin, and simultaneously resorb and secrete the hormones. There do not seem to be different populations of follicular cells.

Cell Proliferation.

In contrast to normal human thyroid, thyrotoxic thyroid shows indirect evidence of significant follicular and stromal cell proliferation (Figs F-2 and F-3). Although serial D.N.A. labelling and cell counts of the type described in Section D, when rats were given a goitrogen to induce cell proliferation cannot be obtained with human tissue there is evidence that cell proliferation must occur in the human thyroid during the development of clinical thyrotoxicosis, and its subsequent treatment; the thyroid typically increases in size and weight 2 - 3 fold and although the follicular cells do hypertrophy (elongate) their number increases simultaneously. For example, both D.N.A. and R.N.A. concentration of thyrotoxic gland are high (Goldberg, Goudie and Ayre 1968). It is, however, not possible because of the lack of suitable fresh tissue, to quantitatively estimate parameters such as growth fractions and cell life cycles. What data there is, however, suggests that the follicular cell population of the average sized (50 gram) representative thyrotoxic thyroid would be replaced about every 2 - 3 years, if the patient was left without treatment (Greig 1964, 1965, Wilson 1967). The cell proliferation is undoubtedly brought about by the sustained action of circulating L.A.T.S. which has biochemical effects on human and rodent thyroid equivalent to that of continuous T.S.H. stimulation (McKenzie 1968). Furthermore there is some fairly direct evidence that human L.A.T.S. promotes cell proliferation. In mice repeated administration of L.A.T.S. positive sera from patients with active thyrotoxicosis not only accelerates all aspects of hormonogenesis but also promotes

follicular cell proliferation and thyroid growth (Sharard 1968, Ochi and De Groot 1969). Smith and Greig (1970) have also shown that mouse thyroid D.N.A. cell labelling indices, of the type described in Section D, increase several fold during L.A.T.S. stimulation indicating induced cell proliferation; Sharard (1969) has shown that L.A.T.S. has no destructive effect on the thyroid epithelium.

While no new therapeutic advantage has accrued from investigations of the origin and actions of L.A.T.S. they are slowly providing some insight into the cause of thyrotoxicosis and its course during and after therapy including radiation therapy. Before considering radiation therapy it is first necessary to briefly review what specific treatments for thyrotoxicosis are available so that the problems of therapeutic research may be put into perspective.

Treatment of Thyrotoxicosis - General.

This subject has been well reviewed and updated by Wilson (1967) and Greig (1966), by McGirr and Greig (1968) and in a series of recent editorials (Lancet 1965, 1969, British Medical Journal 1968). The treatment of thyrotoxicosis has also been the subject of informed conference discussions (Solomon, Bennet, Brown, Peter, Pollock and Richards 1968, Werner 1967 and Irvine 1967).

Historical.

From 1900 to the 1920's one of the popular treatments for thyrotoxicosis was a course (several weeks) of external x-ray therapy to the neck (Hayes 1927). It is difficult to assess the dosimetry

(empirical) and efficacy of this treatment in retrospect but it would appear in general that about 3000 rads was relatively ineffective and x-ray therapy was more or less abandoned (Goolden 1964) when partial thyroidectomy became safer with proper pre-operative preparation of patients (Plummer 1923), safer anaesthesia and improved surgical skills. From about 1925 till 1945 radical partial thyroidectomy was the treatment of choice for thyrotoxicosis although some clinics did also continue to employ x-ray therapy particularly in patients not fit for surgery (Pfahler 1942).

Between 1940 and 1945, however, antithyroid drugs (Astwood 1944) and iodine-131 (Hertz and Roberts 1942) therapy were introduced more or less simultaneously as alternative treatments to partial thyroidectomy. As a consequence from about 1950 up till the present time (1970) case selection for drug treatment, partial thyroidectomy or iodine-131 therapy became necessary. Fortunately in the majority of centres in Europe, Scandinavia and the U.S.A. this has been basically similar for up to 20 years. There is available, therefore, a large volume of experience and literature on which to assess the merits and defects of each treatment.

Current Policy.

It is not possible to predict what the natural course of thyrotoxicosis would be without specific therapy. Wilson (1967) believes on the basis of historical data (Sattler 1908, White 1910, Campbell 1921) that up to 30 per cent might run a fluctuating course

and eventually become euthyroid. While this may have applied in the 1900-1930 era it is not certain how the disease would behave today without treatment. It is at any rate common experience that the large majority of patients who appear in hospital have disability requiring active treatment. The broad outlines of current standard treatments and their defects are summarised in Table F-1.

Drug Treatment.

In general patients who present with uncomplicated thyrotoxicosis whose age is less than 40 years and who have a small goitre (50 grams or less) are given antithyroid drugs. The drug of choice in the U.K. is Carbimazole but Propylthiouracil or Methylthiouracil or Methimazole are used too. The drugs are given in relatively large doses till the patient shows definite improvement. Thereafter the dose is reduced gradually to a maintenance dose when the patient is euthyroid. In this group of patients who comprise about 30 per cent of all new thyrotoxic subjects in the U.K. drug maintenance dose is continued for a total of 1½ to 2 years provided the response is maintained and no reactions occur. In a few (about 10 per cent) control is poor and they are then advised to have surgical treatment. Likewise skin rashes, nausea and leucopenia occur in about another 5 per cent and although other drugs may be substituted, this group in general are also advised to have an operation. It has, however, been the experience of most physicians that the large majority of patients selected for long term drug therapy do well while drug

TABLE F-1

Standard Treatment of Thyrotoxicosis (1950 - 1970)

	Drugs	Operation	Iodine-131
New Patients:	<40 years small gland 30%	<40 years Large gland 20%	>40 years Small and large glands Associated disorder 50%
Therapy:	Drugs --- Reaction ---> Drugs --- No operation ---> I_{131} 1st Dose Drugs --- Poor control recurrence ---> Partial thyroidectomy --- I_{131} Multiple Doses		
Defects (10 yrs):	Recurrence - 50% Reactions - 5%	Recurrence - 10% Hypothyroid - 15% Phonation - 10% Parathyroid - 15% Bad scar - 5%	Slow control - 30 - 40% Hypothyroid - 50 - 70%

therapy is continued. Unfortunately up to 50 per cent of these patients become thyrotoxic when drugs are stopped. Most recurrences are seen within two years of stopping drug therapy. Thus after up to 3½ - 4 years of medical supervision half the patients are little better off. This is the chief problem with antithyroid drug therapy.

Partial Thyroidectomy.

In general and in the last 20 years new thyrotoxic patients whose age is less than 40 years and who have large goitres (more than 50 grams) are considered for partial thyroidectomy. This selection, about 20 per cent of all patients, is based on long standing empirical experience that most of these patients either obtain poor control while taking drugs or they nearly always relapse after drug therapy is stopped.

Patients selected for operation are made euthyroid with drug therapy and after a two week course of big doses of potassium iodide (120 mg per day) they undergo operation. Most surgeons traditionally aim at leaving about 10 grams of tissue. As Table F-1 shows partial thyroidectomy even when performed by experienced surgeons has several permanent defects. These are a recurrence of thyrotoxicosis in about 10 per cent, eventual hypothyroidism in about 15 per cent, change in the natural or singing voice in about 10 per cent, partial or severe parathyroid insufficiency in about 15 per cent and a bad scar in about 5 per cent (Fourman 1967, O'Malley and Kohler 1968, Editorial 1966). It is estimated that only about 50 per cent of patients get an optimum result after

partial thyroideotomy (McNeill and Thomson 1968). Recently there have been attempts to improve the results of drug and operative treatment and these are outlined below.

Research into Methods of Improving Drug and Operative Treatment.

Trends in recent and current research directed towards improving the results of drug and operative treatment are summarised in Table F-2. The main research problem with long term antithyroid drug treatment is the identification at a reasonably early stage of the 50 per cent or so patients who ultimately relapse after drug withdrawal. Although in general patients with a small thyroid receding during treatment, and patients with mild disability responding easily to low maintenance doses of drugs are more likely to remain well after drug withdrawal, this is not invariable (Hershman 1966). Alexander, Harden and Shimmins (1966) have used a test procedure which indirectly assesses whether the thyroid has returned to normal control through the pituitary the implication being that if it has not the gland is still under L.A.T.S. stimulation (i.e. the disease is still active). They give the patients T3 for 7 days at the 6 month stage of drug therapy and without withdrawing drugs carry out a 20 minute thyroid uptake test using iodine-132 (H.L. = 2.3 hours) before and after the T3 course. Some patients show thyroid suppression and some do not. If these patients are ultimately followed after drugs have been withdrawn it is found that most who did not suppress recur and most who did suppress remain euthyroid. This may prove to be a useful procedure with which to select patients best fitted to continue long term drugs. The patients who do not suppress at 6 months may well

TABLE F-2.

Research into Treatment of Thyrotoxicosis

(Potential Reduction of Morbidity).

A. Long Term Antithyroid Drug

Thyroid Suppression test at 6 months

1. Suppression - recurrence less likely.
2. No suppression - recurrence likely.

B. Partial Thyroidectomy

1. Serum thyroglobulin antibody
2. Serum microsomal antibody 1:32
3. Gland lymphoid infiltration

} Hypothyroid risk 25%

4. Radical operation - hypothyroid risk = 20%
   - phonation risk = 10%
   - parathyroid injury = 30%

} Linked.

∴ Less radical operation.

benefit best in the long term from more definitive treatment such as operation or iodine-131 treatment. Trials based on this approach are currently in progress.

Partial Thyroidectomy.

Some of the undesirable consequences of partial thyroidectomy must reflect a number of indefinable factors such as variable care in case selection, different anaesthetic and surgical skills, unanticipated technical difficulties and bad luck. Nevertheless recent observations suggest that some of the complications can be anticipated, prevented, or avoided. For example, when there is serological evidence before operation of autoimmune thyroiditis (positive tests for destructive serum antithyroid antibodies) or after operation (lymphoid infiltration in the thyroid) then the risk of hypothyroidism is increased to about 25 per cent (Irvine and Stewart 1967, Hargreaves and Garner 1968). Patients at risk must obviously be followed or given Thyroxine (0.2 mg. per day) prophylactically.

There is also evidence that the traditional partial thyroidectomy is probably too radical (Fourman 1967). As far as can be determined very radical operations in skilled hands do lead (not surprisingly) to a linked higher risk of hypothyroidism (too much tissue removed), to phonation problems (local trauma, recurrent laryngeal nerve and superior laryngeal nerve damage) and to parathyroid insufficiency (biochemical or clinical). Since less radical operations may not lead to a higher recurrence rate there is a case for a change in surgical technique perhaps including less comprehensive ligaturisation of thyroid and parathyroid

vessels (Green and Wilson 1964, McNeil and Thomson 1968).

Iodine-131 Therapy.

Table F-1 shows that about half of all new thyrotoxic patients are currently considered for iodine-131 therapy but the results are relatively unsatisfactory, the rate of control being comparatively slow yet the hypothyroid incidence is very high. It is now opportune to consider iodine-131 therapy in detail and to briefly discuss the different ways in which these problems are being tackled by other investigators so that the radiobiological and therapeutic research described in this thesis may be placed in perspective.

It has been traditional in the last 20 years not to give iodine-131 therapy to patients less than 40 years of age (Macgregor 1957, 1960) because of the potential risk of late thyroid cancer, leukaemia and genetic damage to the reproductive organs. Although the latter three fears have not been realised (Pochin 1960, Means, De Groot and Stanbury 1963, Thoma, Saenger and Tomkins 1967) the age limit is maintained unless drug treatment is very unsatisfactory, operation is refused or hazardous or the patient suffers from another disorder which limits his/her longevity. About 10 per cent of patients treated with iodine-131 fall into the latter groups. The basic aim with iodine-131 therapy has been to make the patients euthyroid with one dose but to avoid overtreatment (Chapman and Maloof 1955, Blomfield, Eckert, Fisher, Miller, Munro and Wilson 1969). In general, however, and irrespective of how carefully

the initial dose is calculated, the "one dose cure" approach has resulted in two defects one relative, the other absolute. The relative defect is slow control (compared to drug therapy) which is due to a slow response even to an ultimately effective single dose and to the need for retreatment in about 30 to 40 per cent of patients. The absolute defect is permanent hypothyroidism in up to 50 per cent of patients after 10 years, 70 per cent after 15 years and perhaps higher with prolonged and standardised follow up (Beling and Einhorn 1961, Stanbury and De Groot 1964, and Dunn and Chapman 1964, Greig 1964, 1965, Crooks 1965, Smith and Wilson 1967).

Therapeutic Research.

The background thinking and the early and intermediate results of recent investigations to improve patient care are given in a series of papers (Philp, Duthie, Crooks 1968, Barker and Bishop 1969, Smith and Wilson 1967, Golden and Fraser 1969, Hamburger and Paul 1968, Hagen, Ouellette and Chapman 1967) and in Editorials in the New England Journal of Medicine (1967 and 1968). The objectives of each study are to use iodine-131 in a way which achieves cure of thyrotoxicosis simply but without uncorrected hypothyroidism.

It is generally undesirable to control thyrotoxicosis slowly. It would appear that if iodine-131 is to be used alone and to be consistently effective it must be given to deliver rad doses of the order of 10,000 rads (Blomfield et al. 1969). These doses, however, inevitably lead to a high and rising hypothyroid incidence (Smith and Wilson 1967). The latter means that all patients treated with

with iodine-131 must be kept under life-long medical surveillance and supervision. About 400,000 patients have been treated in the past 20 years and many are continuing to be so. Obviously the potential morbidity and the problems of the logistics of follow-up are formidable. Some investigators (Philp, Duthie, Crooks 1968, Barker and Bishop 1969) are attempting to economise follow-up by automated methods the patient attending hospital only when action is mandatory. They use a computor programmed to act on questionaire data provided by post from the patient and his/her family doctor. In some of these systems the doctor also sends a blood sample for a laboratory test such as the serum P.B.I. With this approach only patients suspected of thyroid ill-health are recalled to the clinic for further assessment. While this method is of practical value in the follow up of patients treated in the past, it does not absolve us from the problem of how to deal with patients in the future. The fact that 20 per cent of patients on long term replacement Thyroxine treatment do not adhere to medical advice is justification alone for renewed endeavour and attempts are being made to scrutinise the fundamental defects in dosimetric and radiobiological terms.

Relation Between Iodine-131 Dosage and Results.

The traditional practice has been to decide, from previous empirical experience, the order of mean thyroid iodine-131 rad dose required to give a reasonable chance of remission and then in each patient to calculate and prescribe a mci oral dose (Crooks, Buchanan, Wayne, Macdonald 1960, McGirr, Thomson and Murray 1964).

The mci dose is usually arrived at by clinical measurement of thyroid mass (palpation) by measurement of thyroid uptake of the preliminary tracer test the data being substituted in a mean dose calculation formula of the type:

Mean Thyroid rads per oral mci dose is proportional to:

$$\frac{\text{Uptake (mci)}}{\text{Mass (G)}} \times \frac{\text{Physical Constant}}{1} \times \frac{\text{Te (days)}}{1}$$

The factor Te is usually assumed to be a constant for dose calculation but sometimes it is measured in the preliminary tracer test (Bromfield et al. 1959). The rate of control is variable but the mean of many patients is related to the mean rad dose decided on or the mean mci doses given. The rate of control is for example quicker if it is aimed at giving 14,000 rads instead of 7,000 rads and it is very slow and very often ineffective after 3,500 rads. After the latter up to 60 per cent of patients require supplementary antithyroid drug treatment (Smith and Wilson 1967).

The slow rate of control of thyrotoxicosis with doses below 10,000 rads has been shown to be accounted for by two related factors. One is that the lower the initial dose, the more often is retreatment at 3 - 4 monthly intervals required; the other is that the lower the initial dose the slower the response even when repeated dosage is not given. For example, when it is aimed to give 7,000 rads the order of dosage used in the U.K. in the 1950 - 1960 decade, it was found that about 30 - 40 per cent of the patients required a second dose and up to 10 per cent 3 or more doses. In this group the response rate was slower than could

RADIOACTIVE IODINE (I_{131}) ALONE.

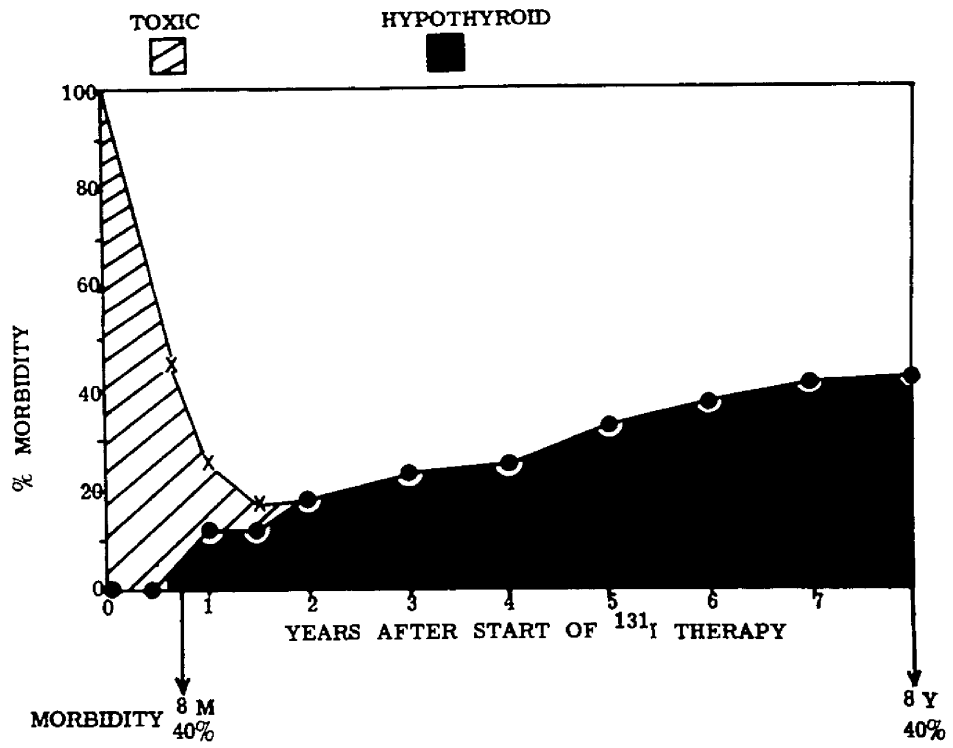


Fig. F-6 Summary progress of 242 thyrotoxic patients treated with iodine-131 only (adapted from Greig 1966). Note slow control phase yet rising hypothyroid phase.

readily have been achieved with antithyroid drug therapy (Greig 1966, Greig, Crooks and Macgregor 1966, Greig, Aboul-Khair, Mohammed and Crooks 1965). However, in this series reviewed by myself in 1964 the hypothyroid rate was very high and rising. This is shown in Fig. F-6. The latter shows the rising hypothyroid line up to 8 years. It is estimated that up to 90 per cent of patients might be hypothyroid after 25 - 30 years but of course many patients die of natural longevity or other causes before this time interval is achieved (Greig 1965).

Different clinics have in retrospect compared the slope of the hypothyroid graph with the aimed thyroid dosage. When the slopes are compared for different aimed thyroid dosages a crude co-relation has been found, the larger the mean dosage the steeper the slope; it is, however, important that the long term slope exists even after comparatively small doses. Basically the objectives of recent trials, with the exception of large ablative doses of iodine-131 plus prophylactic thyroxine are to control hormonogenesis and to leave viable thyroid follicular cells using antithyroid drugs too when necessary. It is not appropriate here to review all the clinical imperfections of these trials being conducted by other investigators (Editorial - 1967 in New England Journal of Medicine). It is, however, relevant to examine iodine-131 therapy as a special radiobiological problem by analysing RELATIONS between iodine-131 irradiation, cell hormonogenesis and cell proliferation of thyroid cells <u>in vivo</u> together with microdosimetry. The studies in rats described in this thesis are helpful in this respect.

THE OBJECTIVE OF RADIATION TREATMENT FOR THYROTOXICOSIS.

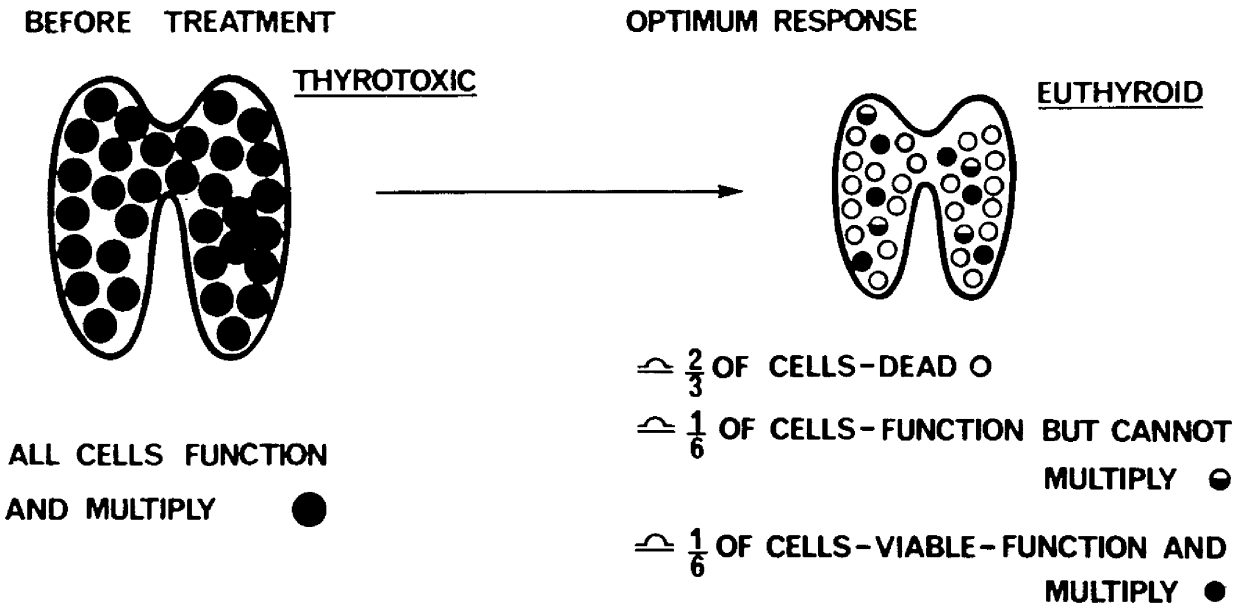


Fig. F-7 Diagrammatic view of radiobiological objectives in radiation (iodine-131) treatment of thyrotoxicosis. Aim is to leave totally viable cells. (From Greig 1966).

Iodine-131 Therapy - Radiobiological Objectives.

The thyrotoxic thyroid gland is composed of discrete populations (follicles) of follicular cells all apparently viable in respect of hormonogenesis and reproductive integrity. The radiobiological aim of iodine-131 therapy is to reduce but not abolish the hormonogenetic capacity of the follicular cells and simultaneously to conserve viable cells in respect of reproductive integrity. This aim can be depicted in a diagram (Fig. F-7). Fig. F-6, however, shows that if iodine-131 is used alone even in conservative doses (40 per cent of patients needed 2 doses) there is still a high prevalence of hypothyroidism, which rises steadily with time. Fig. F-6 itself, can be viewed as the result of a radiobiological but therapeutic experiment. The graph (Fig. F-6) shows features which can be interpreted as consistent with the hypothesis that human thyrotoxic thyroid cells show responses to radiation similar to those observed in rats (Greig 1965) and proven in Sections C, D and E respectively. In rats all the hormonogenetic functions of the thyroid were shown to be relatively resistant to radiation injury (Section C) and the relative resistance of thyrotoxic cell hormonogenesis to radiation suppression is seen in the dosage necessary to render patients euthyroid and to some extent the time it takes to make thyrotoxic patients euthyroid.

The hypothyroid line (Fig. F-6) rising steadily and annually after suppression of mean cell hormonogenesis must be taken to imply that not only were the follicular cell populations homogeneously irradiated but that additional injury was of a type which also

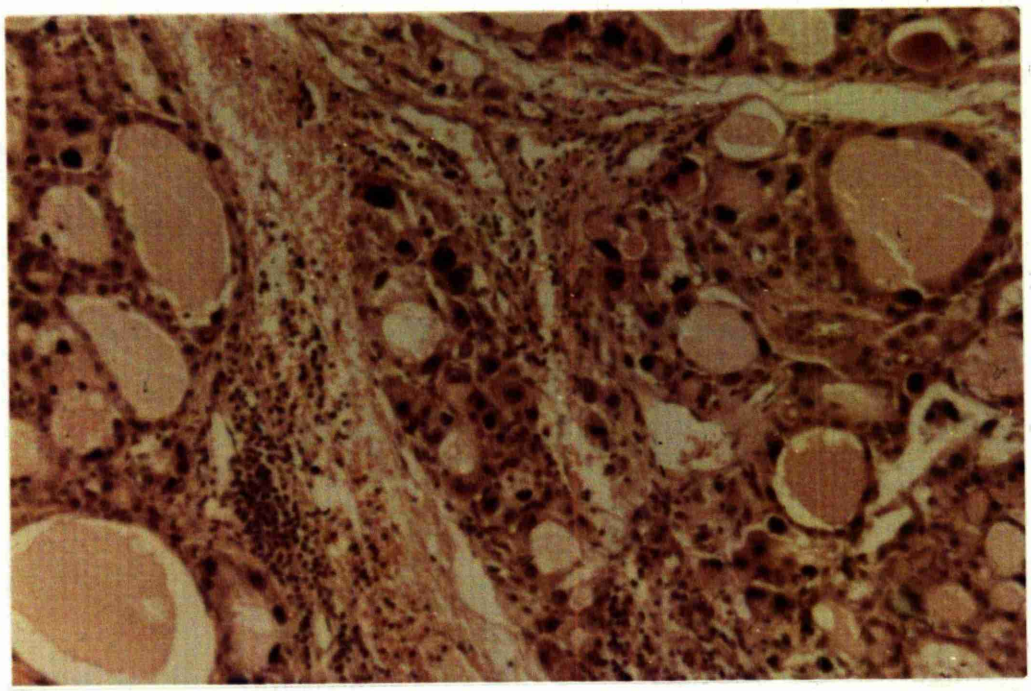


Fig. F-8 Light microscopy of residual thyroid tissue from patient euthyroid 5 years after iodine-131 treatment. Note disorganisation, nuclear abnormalities and vascular damage.

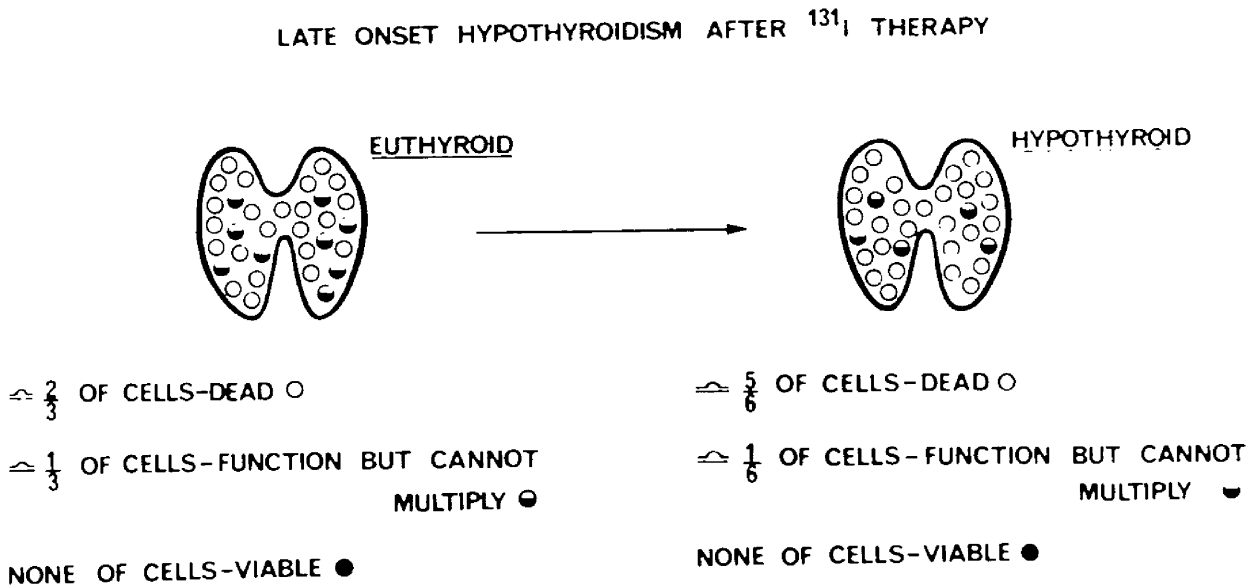


Fig. F-9 See also Fig. F-7. Diagrammatic view of radiobiological events in change from euthyroidism to hypothyroidism, after iodine-131 therapy. No viable cells survive. [FROM GREIG 1966]

also prevented cell proliferation or renewal. Support for this is found in the cytological evidence of nuclear injury to follicular cells in irradiated thyrotoxic thyroid. The nuclei are abnormal and large (Dobyns and Didtschenko 1961) suggesting cell death at the phase of D.N.A. synthesis (Al-Hindawi and Wilson 1965) and as demonstrated in Section D in this thesis. An example of irradiated thyrotoxic gland is shown in Fig. F-8. As indicated earlier in this section (F) the thyrotoxic gland under stimulation by L.A.T.S. is in some ways equivalent to the goitrogen or T.S.H. stimulated rat thyroid model (Sections D and E).

The rising hypothyroid rate after iodine-131 therapy (Fig. F-6) can therefore be interpreted radiobiologically as a progression of cell divisional impairment resulting in failure of cell renewal (Fig. F-9). As demonstrated in Section D differentiated rat thyroid cells appear to have a divisional capacity limited to 2 or 3 divisions and after irradiation this capacity is further impaired. If, therefore, the thyrotoxic follicular cell population has a similar intrinsically limited divisional capacity which is further impaired by iodine-131 irradiation the rising hypothyroid graph probably represents increasing numbers of the residual cells reaching their natural divisional capacity, or it may mean that gland cell populations which have different mean generation times become hypothyroid at different rates. The most likely explanation, however, is that the rising line of hypothyroidism represents a spectrum and a

TABLE F-3

Summary of Iodine-131 Doses in Thyrotoxic Gland

Microscopic

1. β-rays (500 u) $\gg$ cell height (10-20 u)
   Nuclear (division) dose = cytoplasm
   (hormone synthesis dose)

Macroscopic

2. β-rays (500 u) $>$ follicule diameter (100-200 u)
   a) Mutual irradiation of follicles
   b) Irradiation to stroma

i.e. Rad dose distribution is homogeneous.

Summary of Radiobiology of Iodine-131 Therapy

1. Hormone synthesis (cell cytoplasm) is radioresistant
2. Cell division (cell nucleus) is radiosensitive
3. I-131 therapy depresses hormone synthesis and sterilises cells
4. Cell renewal in remnant ceases and hypothyroidism occurs [FIG F-9]

combination of variables including initial mean dosage, cell divisional capacities, generation times, size of post-radiation but injured population, and vascular injury. There does not appear to be any progressive autoimmune destruction of thyroid tissue after irradiation if judged by antithyroid antibody titres or thyroid histology (Irvine, Macgregor, Stuart 1962, Irvine and Stewart 1967, Einhorn, Fragraeus and Johnsson 1965) so that widespread radiation injury to cell renewal seems to be the dominant lesion. Thus the radiobiological analysis, so far, leads logically to the conclusion that in most thyrotoxic thyroids the iodine-131 dose distribution is homogeneous too. A brief consideration of iodine-131 microdosimetry in the thyrotoxic thyroid would support this supposition.

Iodine-131 Therapy - Microdosimetry.

The rad dose distribution in the thyrotoxic gland is nearly homogeneous across and through the follicular cells (Table F-3). The colloid location of iodine-131, as in the rat (Section B), confers no dosimetric and/or radiobiological advantage in terms of differential high dosage to the resistant hormone synthesising parts of the follicular cells and low doses to the cell nuclei. This is because the range of iodine-131 β-rays, average 500 um, in the gland is much greater than the follicular diameters. As a consequence the absorbed rad doses are the same through the follicular cell apices, body of cells and nuclei. In addition mutual irradiation between several contiguous follicles results in the radiation doses being generally homogeneous and

the doses to the intrafollicular stroma being as high as those within the follicles and through cells (Gillespie, Orr and Greig 1970). As a result the doses to almost all microscopic parts of the gland will be very similar and more or less equal to the mean gland dose (Table F-3).

These considerations also indicate that the mean doses to individual follicular cells after any given mci dose of iodine-131 are determined by total gland mass (colloid plus follicular cells plus interfollicular stroma) in addition to thyroid uptake and effective half-life (Te). This implies that accurate iodine-131 dosimetry (mean and microscopic doses) makes an accurate measurement of total gland mass mandatory. So far, however, not even precise scanning measurements, or nomograms, have provided accuracy, in this respect, of better than ±20 per cent (Renda, Holmes, North and Wagner 1967, Means, De-Groot and Stanbury 1963, Myhill, Reeve and Figgis 1956, Himanka and Larsson 1955). Furthermore, solutions to measuring other variables as they affect the therapeutic dose are not perfected. For example, there are variable differences between the tracer uptakes in different patients and between tracer uptake and its Te and those parameters of the therapy doses in the same patients.

While inaccuracy of iodine-131 mean dosimetry might, somehow, be minimised with more careful measurements, the microscopic dosimetric problems appear to be unavoidable, because whatever the mean iodine-131 dose is in the gland, the absorbed doses through the follicular cells is always homogeneous. As a result the

TABLE F-4

Iodine-131 Therapy: Theoretical Modifications

1. Make microscopic or macroscopic dose distribution irregular - How?
2. Make hormone synthesis less radio-resistant - How?
3. Make cell division less radiosensitive - How?
4. Protect stroma - How?

doses to the hormonogenetic parts of the follicular cells are always equal to the doses to the nuclei. Since cell hormonogenesis is radio-resistant (Section C) compared to cell reproductive integrity (Sections D and E) it follows that even if the mean dosimetry was very accurate the doses given to depress hormonogenesis would always result in damage to cell nuclei, be great enough to virtually abolish cell renewal and these changes are homogeneous throughout the thyroid. It follows, therefore, that to avoid hypothyroidism after iodine-131 therapy one of several theoretical modifications at a cellular level should be considered and it is worth examining their practical feasibilities.

Iodine-131 Therapy - Theoretical Modifications.

These are summarised in Table F-4. If some simple procedure could be found whereby the dose distribution of iodine-131 would be much more inhomogeneous and the inhomogeneity predictable, control might be feasible without organ destruction. This might be macroscopically (more dose to one follicle than to another follicle) or microscopically (more dose to the radio-resistant hormonogenetic parts of the cells than to the divisional parts (nuclei). There does not, however, appear to be any simple and safe method whereby this could be achieved accurately and consistently enough in practice.

It is feasible that if some means could be devised whereby, without making mean dosimetry more inaccurate, the hormonogenetic parts were made less radio-resistant that thyrotoxicosis could be controlled with small mean doses of iodine-131. If this were

possible the nuclear doses would be less, and the interfollicular tissue doses less too. In these circumstances, it is probable that the hypothyroid rate would be lowered (Smith and Wilson 1967). Unfortunately no method is available with which to manipulate radiosensitivity in this way and the same comment applies to the alternative possibility which is to make follicular cell nuclei less radiosensitive. If injury to the interfollicular stroma does play a contributory part in organ failure then a reduction in dosage to stroma or reducing its radiosensitivity might be helpful. There are, however, no methods of achieving this.

Comments on Use of X-ray Therapy.

The problem of homogeneous dosage from iodine-131 can be circumvented if external X or γ rays, and positive shielding are employed, but nobody has attempted this. Trotter and Willoughby (1967) have, however, suggested the use of focal external X-irradiation in a dose which will suppress subsequent iodine-131 uptake in that part of the gland while the uptake in the remainder is still high. This approach would, however, appear to have no advantage since the problem of leaving a non-irradiated cell population is not solved. Philp, Harrison, Ridley and Crooks (1968) have given external X-irradiation uniformly to the whole gland in doses, which in crude rat cell survival studies (Philp 1966), would be expected to leave a proportion of intact cells. They found it necessary, however, to use antithyroid drugs in some of their patients to control persistent thyrotoxicosis and the long term results are not yet known.

In my opinion and that of others (Hulbert 1969), however, the use of external X-irradiation in the treatment of thyrotoxicosis is not justified. My principal objections are the undoubted risk of post-cricoid and nasopharyngeal cancer, damage to parathyroid glands, inconvenience to the patient, and the possibility of creating a cancer phobia (Slaughter and Southwick 1957). It is of interest, however, that doses of X-irradiation, up to 900 rads, were relatively ineffective in the control of thyrotoxicosis (Philp et al. 1968) and this is compatible with the relative radio-resistance of hormonogenesis in the rat thyroid after similar doses of external x-rays (Section C) or in the normal human thyroid during the treatment of laryngeal cancer (Greig et al. 1965).

It thus seems that the only remaining approach to radiation treatment of thyrotoxicosis is to irradiate the hormonogenetic parts of the follicular cells more than their nuclei and this is the potential advantage of iodine-125.

Iodine-125 Treatment for Thyrotoxicosis - Rationale, Dosimetry and Trials.

Rationale Behind Use of Iodine-125.

As shown in Section B iodine-125 irradiates the apical hormonogenetic parts of the normal rat follicular cells more than the nuclei and the dose to the interfollicular stroma is the lowest in the gland. This differential radiation arises because of the colloid location of iodine-125 and the abundance of dose which is due to large numbers of electrons with energies so low that their ranges into the surrounding follicular cells are much shorter than

TABLE F-5

Summary of Principal Electron Radiations from Iodine-125

Electrons

Per 100 disintegrations	Range in tissue
No.	um
376	< 0.05
234	< 0.50
24	< 15.00
16	< 30.00

Iodine-125:

1. Irradiates hormonogenetic parts of cells more than nuclei (division)
2. Irradiates stroma at low dose
3. Irradiates one follicle more than another

Theoretically thyrotoxicosis controlled without hypothyroidism.

the average distance between the colloid cell boundary and the cell nucleus; most of the iodine-125 electrons have ranges less than 0.50 um. In the average rat thyroid of 20 mg, 50 per cent of volume colloid, and with 5 um cuboidal follicular cells the total (electron plus x-ray) nuclear doses are at least half of those to the apical membrane where the critical stages of thyroglobulin iodination, maturation and resorption take place (see Fig. B-6).

These concepts and calculations, discussed in detail in Sections A and B, were linked together with the radiobiological data (Sections C, D and E) to formulate the rationale for iodine-125 therapy (Table F-5). It was shown in Section C that although hormonogenesis was radio-resistant this was only relative since it could be progressively impaired by increasing doses of irradiation and this applied to the iodine-125 studies too. Furthermore, it was shown in Sections D and E that it appears to be nuclear damage which stops cell division in the thyroid as also suggested by Speight, Baba and Wilson (1968). For example, compared to X-irradiation and iodine-131 irradiation greater mean doses of iodine-125 were required to impair cell division because with iodine-125 the nuclear doses were lower than the mean gland doses. Since the iodine-125 doses to cell nuclei are lower than the doses to the hormonogenetic parts of the cells it appeared that iodine-125 might be useful as a substitute for iodine-131 in the treatment of thyrotoxicosis. In fact many of the structural and functional features of the thyrotoxic thyroid appear to enhance

the potential advantages of iodine-125 in this respect. Compared to the average rat thyroid, the average human thyrotoxic thyroid contains less colloid (about 15 per cent of the volume) much longer follicular cells (15 - 30 um) and the cell nucleus (4 x 3 x 3 u) is at the basal end of the cell (see Fig. F-2). These changes lead to a steeper fall in the iodine-125 electron dose across the apical cell margin and apical segment of follicular cell and therefore to a greater difference in dose between this part of the cell and the cell nucleus. Furthermore the doses to the interfollicular stroma are lowest in the gland and mutual irradiation of follicles is minimal. If to this is added the theoretical possibility that the increased hormonogenesis and activity at the cell membrane margin (Myers and Karazin 1968) render this structure more radiosensitive than in the rat, while the cell nuclear radiosensitivity is unchanged, then it is feasible that doses of iodine-125 might control thyrotoxicosis without post-radiation hypothyroidism. Since the use of iodine-125 retains the advantages of iodine-131, convenience and simplicity, it seemed worthwhile planning preliminary therapeutic trials as originally suggested by Greig (1968).

Iodine-125 Trials - Dosimetry in Thyrotoxic Thyroid.

In Section B a detailed account was given of the iodine-125 decay characteristics (Tables B-2 and B-3) and the electron dose distributions from point sources (Fig. B-3) from plane sources of infinite and finite thickness (Fig. B-4) from spheres of different diameter 35 um and 70 um respectively (Fig. B-5). In addition the dose distribution in a normal rat thyroid and particularly

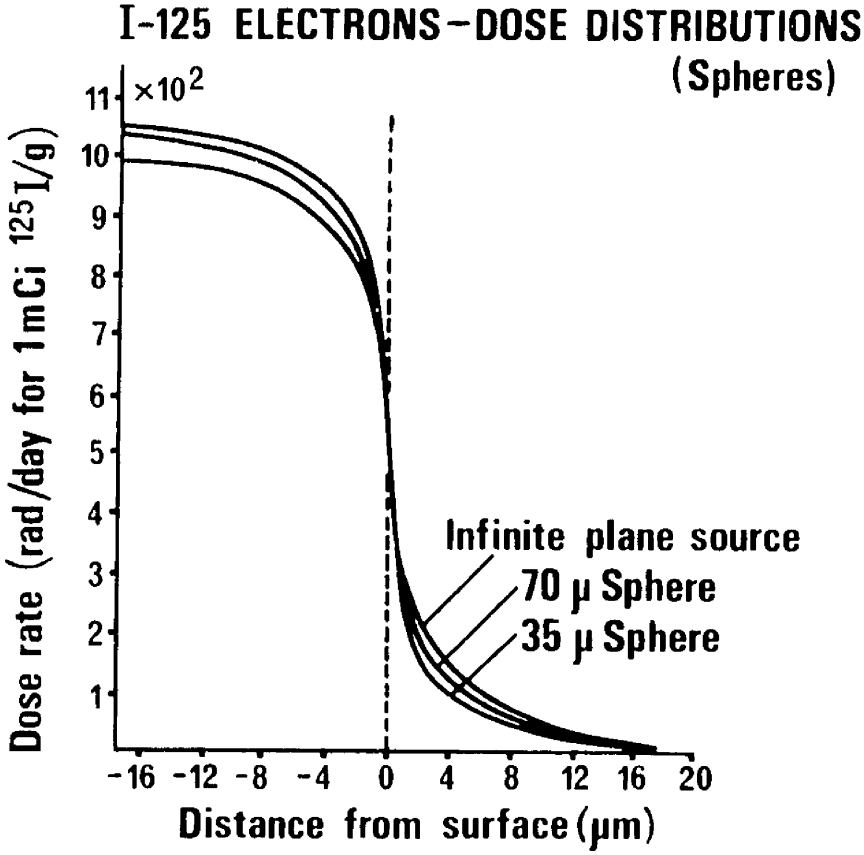


Fig. F-10 This is also Fig. B-5. Electron Dose distributions due to two uniform spherical sources and an infinite plane source of iodine-125 (1 mci per gram).

the dose fall off across the surrounding follicular cells was given (Fig. B-6). It is not necessary here to repeat the mathematical, biological, and experimental data behind Figs. B-3 to B-6, but the electron dose distribution about two spheres of 35 and 70 um respectively and compared to that from an infinitively thick plane source is shown again in Fig. F-10. This demonstrates that for equal concentrations (1 mci per gram) of iodine-125, the electronic dose rates and their dose distributions are similar for each of the three geometries. This is especially relevant to the predicted dosimetry of iodine-125 within the thyrotoxic thyroid because the colloid lumina in untreated thyrotoxicosis shows a variety of diameters and shapes (see Fig. F-2). Thus in the thyrotoxic gland the rapid fall off in electronic dose rate within a few um of the colloid boundary and apical membrane is likely not to be affected by shape of the lumina alone. It is possible, however, that the character of the fall-off in electron dose might be affected by the proportion of the gland iodine which is the colloid and that which is in the cells. Using the same physical data employed for Fig. F-10 and taking for a model, a thyrotoxic gland of 25 grams with 15 per cent of its volume as colloid, calculations of doses (electronic and x-ray) within and about a follicle of radius 35 um were made for two situations. These were where all iodine-125 was in the colloid and none in the cells and where 90 per cent of the iodine-125 was in the colloid and 10 per cent was in the cells. The dose characteristics are shown in Fig. F-11 which includes the

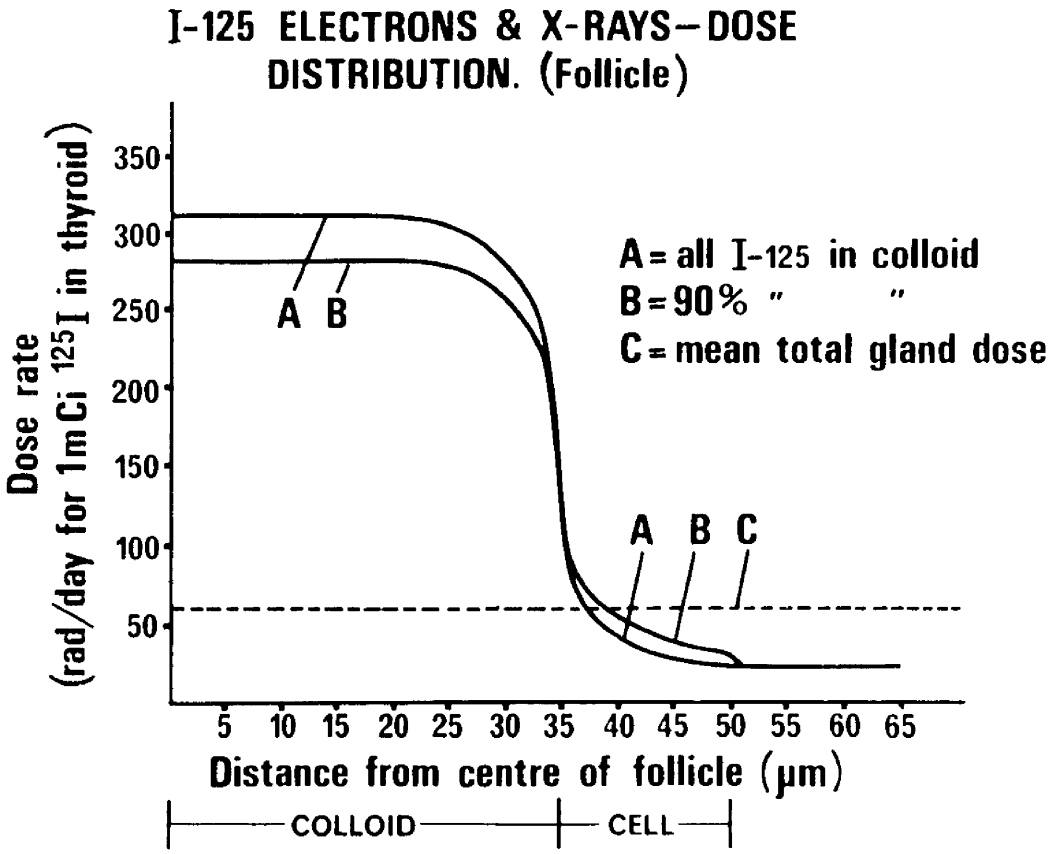


Fig. F-11 Total dose distributions around a 70 um spherical follicle in a 25 g thyrotoxic gland. Colloid volume taken to be 15 per cent of gland volume. Curve A is result for all of the iodine in colloid. Curve B for 90 per cent in colloid, 10 per cent in follicular cells. Curve C is mean total gland dose.

contribution from x-rays and therefore refers to total doses and also shows the mean total iodine-125 dose throughout the gland as a whole; the data is for the dose-rate in rads per day arising from 1 mci of iodine-125 in the 25 gram thyroid but without biological loss. It is clear from Fig. F-11 that there is little difference between the dose inhomogeneity both dose rates falling rapidly to the mean thyroid dose within 3 um of the colloid edge. Because of the contribution from homogeneous x-rays the dose rates do not fall to zero; nevertheless the dose rates at positions 10 - 15 um from the colloid cell interface (region of nucleus) are substantially less than those up at the interface and less than those to the gland as a whole (the mean dose). As described previously the follicular cells in the thyrotoxic gland have lengths of about 15 - 30 um and their nuclei are placed at the basal ends of the cells. Thus if Fig. F-11 represents the dose distribution in a typical thyrotoxic follicle the dose to the average follicular cell nuclei will be relatively much lower than those to the average apical margins, the difference in dose being of the order of 3 times. Since in the thyrotoxic gland the apical end of the cell margin is actively engaged in thyroglobulin secretion and pinocytic absorption (see Figs. F-4 and F-5) a trial of iodine-125 in thyrotoxicosis seemed justifiable on dosimetric and radiobiological grounds, the iodine-125 delivering the highest doses to the radio-resistant hormonogenetic portions (apices) and the lowest dose to the radiosensitive portions (nucleus).

Pilot Trials of Iodine-125 Therapy.

Pilot Study No.1 Control of Thyrotoxicosis

The degree of radio-resistance of hormonogenesis of the thyrotoxic gland, as determined from experience with iodine-131 therapy, appears to be such that at least 12,000 rads are required to rapidly control thyrotoxicosis predictably. It was, therefore, first necessary to determine from the outset whether iodine-125 given in amounts to deliver about 12,000 rads at around the apical end of the cell was effective or not in controlling thyrotoxicosis beforedefinitive long term trials could justifiably be mounted. Using the same average thyrotoxic thyroid model described for Fig. F-11 and assuming an average T_B of 12 days it was calculated that the mci dose of iodine-125 required would be 3 times those of iodine-131 that would have been used in the same patients; because of the uncertain reduction in R.B.E. due to the slower dose-rate from iodine-125 and because I was specifically examining the dose levels required to control the thyrotoxicosis as a preliminary to planning long term definitive studies the mci amounts of iodine-125 were increased by another unit to 4 times those I would have given if iodine-131 had been used.

Pilot Study No.1

After consultation with the Isotope Advisory Panel of the Medical Research Council, pilot study No.1 was confined to patients over 60 years old, and was made on ten subjects who had overt clinical but uncomplicated thyrotoxicosis which was confirmed by

Table F-6

Control of Thyrotoxicosis with Iodine-125

Pilot Study 1.

Patient (no.)	Approximate gland mass (G)	Tracer uptake (%-24hrs)	Oral I-125 dose (mci)	Weeks till euthyroid
1	60	73	57	6
2	25	59	40	6
3	40	53	40	6
4	25	65	25	6
5	25	61	25	7
6	30	65	24	7
7	50	70	56	8
8	25	99	30	12
9	25	74	25	20
10	50	46	50	9
Means	35.5	66.5	*38.2	8.5

* The mean oral dose of conventional I-131 therapy would have been 10 mci in these patients.

laboratory measurements.

The dose (mci) of iodine-125 administered orally to each patient was four times that of iodine-131 (mci) which on the basis of clinical assessment of gland mass (grams) and a 24 hour uptake (tracer test dose of iodine-131) would otherwise have been given to produce rapid control and early hypothyroidism.

Following the iodine-125 therapy each patient was reviewed at two-weekly intervals. Evaluation of the patient's condition was based on clinical assessment (Crooks, Wayne and Robb 1960) and measurement of serum protein bound iodine (Farrell and Richmond 1961).

Results of Pilot Study No.1

Table F-6 lists the relevent data for each patient. The mean oral dose of iodine-125 administered was 38.2 mci to a mean thyroid mass of 35.5 grams. This compares with the mean dose of approximately 10 mci of iodine-131 that would have been given in my routine therapy to this group of elderly patients. If iodine-131 therapy had been used, control would have been expected on average in most of the 10 patients in about 12 - 16 weeks. But with iodine-125 therapy all 10 patients became euthyroid rapidly, 8 within 10 weeks and the other 2 within 12 and 20 weeks respectively (mean time to become euthyroid = 8.5 weeks).

Pilot study No.1 thus showed that iodine-125 given in administered doses of about 1000 uci per gram thyroid resulted in rapid and predictable control of thyrotoxicosis. The only side effect present in 3 patients and noted 2 to 6 weeks after therapy,

was slight dysphagia and thyroid tenderness. All 10 patients have been followed up for 48 weeks and there has been no change in white cell count and no recurrence of thyrotoxicosis but 20 per cent are now hypothyroid.

Comment on Pilot Study No.1

With the mci doses of iodine-125 employed in the pilot study it was estimated that the average radiation dose to the apical edge of the cells was at least 12,000 rads and possibly as high as 15,000 rads. This would explain the rapid rate of control of thyrotoxicosis and indicates that hormonogenesis is only relatively radio-resistant. As a consequence it became clear that lower doses might give an acceptable rate of control of thyrotoxicosis and in Pilot Study No.2, now to be described, 3 dose levels of iodine-125 were used. One was the same as in pilot study No.1, another was 60 per cent of this and another was 40 per cent of it. The aim was to obtain clinical control within 3 - 4 months of a single treatment in a high proportion of the patients; since the radiation doses to the follicular cell nuclei would be much less compared with conventional iodine-131 therapy, at these dose levels, it was hoped that the hypothyroid risk would be proportionately reduced.

Pilot Study No.2

In this study a total of 50 patients participated; 12 patients (all over 60 years) were given aimed administered doses of 1000 uci per gram thyroid and 18 patients (all over 40 years) were given 600 uci per gram thyroid and 20 patients (all over 40

TABLE F-7

Iodine-125 Therapy - Basic Diagnostic Data

Pilot Study No. 2

Aimed Administered Dose uci/gram	1000	600	400
Number of Patients	12	18	20
Males	2	2	3
Mean Age (years)	65	56	51
Mean Gland Mass (grams)	34	35	41
Mean Tracer Uptake (24 hrs (% dose)	66	57	60
Mean Serum PBI_{131} 48 hrs (% dose per litre)	1.10	0.73	1.24

TABLE F-8

Iodine-125 Therapy - Therapeutic Results

Pilot Study No. 2

	Dose Levels			
Aimed Administered Dose uci/gram	1000	600	400	600 or 400
Number of patients	12	18	20	38
Mean Dose Given (uci/gram)	1056	567	407	483
Euthyroid with one Dose (%)	100	63	85	79
Mean Response Time to Dose I (wks)	9	14	14	14
Mean Follow Time (wks)	40	26	24	25
Hypothyroid (% Total)	25	0	15	8

years) were given 400 uci of iodine-125 per gram thyroid. All 50 patients had overt clinical but uncomplicated thyrotoxicosis confirmed by laboratory tests including preliminary radioiodine tracer tests. The weights of the thyroids were estimated by clinical palpation. After treatment they were examined frequently and change in clinical state was checked by estimation of serum protein bound iodine. The study was conducted with the permission and advice of the Isotope Advisory Panel of the Medical Research Council.

Results of Pilot Study No.2

The results are summarised in Tables F-7 and F-8 respectively. Table F-7 shows the basic diagnostic information, and indicates that the means of the items gland mass, tracer uptake (24 hours) and tracer protein bound radioiodine (48 hours) were comparable in the three treatment groups.

Table F-8 shows the therapeutic data analysed after follow-up. It shows that in the "high dose group" which was a reproduction of pilot study No.1 (aimed administered dose 1000 uci per gram) the mean dose actually given was 1056 uci per gram. In this group the results were almost the same as for the pilot study No.1 (Table F-6). All the patients (100 per cent) became euthyroid with one dose, the mean time taken being only 9 weeks. After a follow-up time of 40 weeks, however, 25 per cent had developed hypothyroidism.

In the "intermediate dose group" (aimed administered dose of 600 uci per gram thyroid) 63 per cent became euthyroid with one dose. The remaining 37 per cent were given a second mci dose of

iodine-125 which was numerically equal to the first dose. All these patients became euthyroid after the second dose. In this group the mean time to become euthyroid, when it was achieved with one dose, was 14 weeks. After a mean follow-up time of 26 weeks none of the patients (treated with one or two doses) was hypothyroid.

In the "low dose group" (aimed dose of 400 uci per gram) the mean dose given was 407 uci per gram thyroid. With this dose level 85 per cent became euthyroid with one dose, the mean response time to one dose being 14 weeks. The remaining 15 per cent required a second mci dose of iodine-125 which was numerically equal to the first; all of them responded to the second treatment. In this group of patients followed for a mean time of 24 weeks 15 per cent became hypothyroid.

These data (Tables F-7 and F-8) show the paradox that the 20 patients given an estimated mean dose of 407 uci per gram thyroid responded more consistently than the 18 patients given 367 uci per gram thyroid, the response rate to one dose being 85 per cent and 63 per cent respectively but the mean response times were the same (14 weeks). The hypothyroid rates, at equivalent follow-up times, were 15 per cent and 0 per cent respectively. The explanation behind these anomalous responses are not considered to be biological since the patients were comparable (Table F-7) but to be dosimetric. By this is meant that the doses were often inappropriate perhaps arising through differences in colloid volumes in the thyroid; the importance of colloid volume is discussed in detail below.

If the data for the 600 uci and 400 uci groups are combined

TABLE F-9

Iodine-125 Therapy - Summary

Pilot Study No. 2

Number of patients	12	38
Mean Gland mass (grams)	34	38
Mean Dose given (uci/gram)	1056	483
Euthyroid with one Dose (%)	100	79
Mean Response Time (wks)	9	14
Mean Follow Time (wks)	40	25
Hypothyroid (% Total)	25	8
Recurrence (% Total)	0	0

empirically it is found that the mean dose given to the total of 38 patients was 483 uci per gram (i.e. mci doses of the order of 15 - 20 mci). This is shown in Table F-9 which also shows that 79 per cent of patients responded to one dose (all the others responded to the second dose) in a mean time of 14 weeks. In this combined group, the hypothyroid incidence after a mean follow-up of 25 weeks was 8 per cent. Recurrence of thyrotoxicosis, after control, was not noted in any patient. Four of the total of 50 patients complained of slight dysphagia at about 3 weeks after the dose was given and in 2 patients there was some tenderness of the thyroid region on palpation at this time. Serial peripheral blood (white cell counts) carried out over the length of follow-up showed no change.

Comment on Pilot Study No.2 and Plans for Definitive Dosimetric Trials.

This study shows that the order of administered doses of iodine-125 which give control, comparable to that of iodine-131, lie in the region 400 - 600 uci per gram thyroid and doses of 1000 uci per gram are unnecessarily high. The anomalous responses between the 400 uci and 600 uci group (Table F-8) demonstrate, however, that in definitive trials being planned much more attention must be given to dosimetry. In the pilot studies, the doses given were crudely prescribed on the basis of a clinical estimate of gland mass only. The error of the latter is probably about ±20 per cent. In addition no account was taken of the time-

phase pattern of uptake of the iodine-125, the biological life of thyroid iodine-125. In the definitive trials which are now being planned it is intended to prescribe the dose of iodine-125 on a much more individual patient basis. This will entail a detailed examination of the behaviour of the tracer dose (uptake phase $T_{\frac{1}{2}}$, maximum uptake (percentage dose), and loss phase $T_{\frac{1}{2}}$; in addition using accurate thyroid scanning it is intended to get a more consistent index of gland mass (computed from nomograms of thyroid shape and scan area). These data will be used to calculate the order of mci doses of iodine-125 required to deposit 600 uci per gram thyroid and 400 uci per gram thyroid in alternate patients. The actual mci doses given will, however, also be adjusted for a "colloid mass factor".

Colloid Mass Factor.

Most or all (90 per cent or 100 per cent) of the iodine-125 is located in the colloid and most of the electronic irradiation dose is given to a few um outside the colloid to the inner portions of the follicular cells (Fig. F-11). Since the average colloid "radius" is 35 um and the average of half the length of the surrounding follicular cells is 7.5 um, it is clear that it is the colloid mass which determines most of the dose to the follicular cells (and especially to their hormonogenetic parts) and not the total gland mass. With iodine-131 therapy, colloid mass is not critical for cell rad dose determination because the long range β rays (90 per cent of the dose) irradiate the whole gland homogeneously and it is gland mass which is therefore relevant to iodine-131 absorbed cell

dose, as discussed previously. Thus with iodine-125 therapy a "colloid mass factor" must be introduced to dose calculation procedure. Currently some simple means whereby this may be determined *in vivo* before therapy is given are under consideration. For example, it may be possible, using the kinetics of the tracer iodine-131 dose to measure serial thyroid uptake and serial P.B.I.-131 and with a scan index of thyroid mass to obtain a measure of the colloid mass as a fraction of the gland mass (because most or all of gland iodine is in colloid) and thereby to finally calculate the administered dose to deposit 600 uci or 400 uci to each gram of follicular spheres.

If the colloid mass and gland mass can be measured accurately (or with the same error in different patients) it will not be too difficult to decide on the value of the "colloid mass factor" for dose calculation. This is because the expected dose rates to different critical parts of the gland can be precalculated for a range of gland masses and for a range of colloid fractions. For example, assuming a mean effective colloid diameter of 70 um separate results were calculated for the dose rates arising in glands from 20 to 100 grams total mass with colloid fractions from 5 to 20 per cent. The calculations were also made for 100 per cent iodine-125 in the colloid (A) and for 90 per cent iodine-125 in the colloid and 10 per cent in the follicular cells (B). Table F-10 shows these data. For example, if total gland mass was 40 grams and colloid fraction was 15 per cent, the dose-rate at the colloid cell interface, arising from 1 mci iodine-125 in the

TABLE F-10

Influence of Gland Mass and Colloid Content

on Iodine-125 Dosimetry

Total Dose-Rate Rad day^{-1}/mCi ^{125}I in Gland

Gland Mass		20g				40g			
Colloid Fraction		5%	10%	15%	20%	5%	10%	15%	20%
To Whole Gland	A	75	75	75	75	40	40	40	40
	B	75	75	75	75	40	40	40	40
At Colloid-Cell Interface	A	483	253	176	138	244	129	90	70
	B	463	243	170	133	234	124	87	69
At 1 um from Colloid-Cell Interface	A	173	98	73	60	89	51	39	32
	B	211	117	86	70	108	61	45	38
At 10 um from Colloid-Cell Interface	A	53	38	33	30	29	21	19	17
	B	91	57	46	40	48	31	25	22

TABLE F-10

Influence of Gland Mass and Colloid Content

on Iodine-125 Dosimetry

60g				80g				100g			
5%	10%	15%	20%	5%	10%	15%	20%	5%	10%	15%	20%
28	28	28	28	21	21	21	21	18	18	18	18
28	28	28	28	21	21	21	21	18	18	18	18
164	88	62	49	123	66	47	37	99	53	38	30
158	84	60	48	118	63	45	36	95	51	36	29
61	36	28	23	47	27	21	18	37	22	17	15
74	42	32	27	55	32	24	20	45	26	20	16
21	16	14	13	16	12	11	10	13	10	9	8
34	22	19	16	25	16	13	12	21	14	12	10

gland, would be between 90 and 87 rads per day; or from 25 mci iodine-125 in the gland the dose-rates would be 2250 rads per day. Thus if maximum thyroid uptake was 75 per cent and thyroid effective half-life was 12 days the total doses at the colloid cell interface would be about 20,000 rads. The corresponding doses at a point 1 um from the border would be about 10,000 rads and at a point 10 um from the border (nucleus) would be about 5,000 rads.

The doses to the follicular cell nuclei are, however, largely dependent on the absorbed dose attributable to photon irradiation with little coming from electron dose (see Fig. F-11). Thus in the event of not being able to measure colloid mass (not feasible yet) but being able to accurately measure total gland mass (should be feasible with scan and computer) some index of nuclear dose (the one which will probably determine the hypothyroid rate) should be available if the absorbed doses due to X or γ irradiation only are known by calculation or experiment before dose prescription. An assessment of these doses were arrived at experimentally as follows.

Absorbed Photon Doses in the Thyrotoxic Thyroid.

A relatively large proportion (68 per cent) of the total energy released in the disintegration of iodine-125 is, on average, in the form of low energy photons. However, as is shown below, owing mainly to the size of the thyroid, only a relatively small fraction of this photon energy is actually absorbed in the gland and the fraction varies according to gland size.

Almost all of the 3.1 KeV/disintegration attributable to 3.7 KeV Te L X-rays will be absorbed since the half-value thickness in tissue of 3.7 KeV photons is 0.11 mm. In the range 24.5 KeV to 35.5 KeV, which includes the major photon emissions, the probability of photoelectric absorption in tissue is approximately the same as for Compton scattering. However, in this energy range, the energy of the recoil Compton electron, and consequently the energy deposited locally, is very low (less than 3 KeV) and most (95 per cent) of the photon energy absorbed is attributable to photoelectric interactions. The mean photon energy deposited locally was determined experimentally in the following way.

For each of a series of phantoms of volume between 5 ml and 50 ml the fractional number of photons of energy between 24.5 KeV and 35.5 KeV absorbed in the phantom was measured. The phantoms which were intended to represent thyroid lobes had perspex walls of negligible thickness and were either in the form of spheres or of cylinders with lengths three times their diameters. With each phantom filled with a solution containing iodine-125 at a fixed concentration, the photopeak detection rate in a sodium iodide scintillation probe 1 metre from the phantom was determined. The cylindrical phantoms were monitored with their central axes at various angles to the axis of the probe to allow calculation of the mean detection rate over 4 sterad. For each phantom, the detected rate per unit volume was then calculated. Since the resolution of a sodium iodide scintillation spectrometer

TABLE F-11

Mean energy deposited in thyroid by photons per iodine-125 disintegration for various gland masses

Gland Mass g	Mean Energy Deposited/Disintegration KeV
10	6.8
20	8.8
30	9.9
40	10.6
50	11.2
60	11.8
70	12.3
80	12.8
90	13.3
100	13.8

is not good enough to distinguish scattered from direct radiation at around 30 KeV the expected and observed reduction in detection rate per unit volume of solution, with increasing phantom volume, was attributable entirely to photoelectric absorption.

The results of these measurements reflected the relative numbers of photons absorbed, not the fractions of the total energy absorbed. However, since both the variations in the photoelectric absorption coefficient in the energy range 24.5 - 35 KeV and the abundances of the various photon emissions in that range are known, it was possible to estimate the fraction of the energy absorbed from the fractional number of photons absorbed. For each phantom, the fraction of energy absorbed as estimated above was increased by 5 per cent to allow for energy deposited by Compton recoil electrons and by a further 10 per cent to allow for the contribution that would be made <u>in vivo</u> by photons scattered back on to the thyroid from surrounding tissue.

The total average energy deposited per disintgegration in the thyroid phantoms by photons from iodine-125 is listed in Table F-11 for various sizes of gland. The measurements give no indication of the distribution of photon dose throughout the gland. However, as has been shown by Ellett, Callahan and Brownell (1964) for larger sources, the microscopic and macroscopic dose throughout the gland will be approximately uniform and will be therefore, the doses given to the nuclei of the follicular cells. If the kinetics

of the dose, uptake and loss $T_{\frac{1}{2}}$, and gland mass is measurable it should be possible to calculate the average absorbed photon doses to the gland and presumably to the nuclei arising from X and gamma irradiation only. It is intended to determine these photon doses in the therapeutic trials and to co-relate them with clinical outcome.

Finally, the possibility that some recirculation of iodine-125 will contribute a second phase of thyroid irradiation will be examined by monitoring the kinetics of the therapy doses themselves. This will entail serial measurement of thyroid, blood and body content of iodine-125. The latter will be assessed using a whole body counter. This has the additional purpose of providing more data with which to calculate doses outside the thyroid and particularly those to the haemopoetic system.

Iodine-125 Radiation Doses Outside the Thyroid.

Doses to Tissues in the Neck.

The radiation doses delivered to tissues in the neck by iodine-125 within the thyroid gland are attributable almost entirely to the 38.8 KeV/disintegration emitted by X and gamma rays of energy above 4 KeV. The effective mass absorption coefficient for the relevant emissions in non-cartilaginous tissue is approximately 0.19 cm^2/g. Using this value it may be shown that the maximum dose-rate in non-cartilaginous tissue adjacent to the thyroid lobes from an administered dose of 1 mci iodine-125 is 0.20 rads per hour assuming a gland mass of 25 grams and a peak uptake of 70 per cent. In the same circumstances, the dose-rate

to tissues 1 cm from the surfaces of the lobes is approximately 0.05 rads per hour per mci iodine-125 administered. Assuming further an effective half-life of 12 days of iodine-125 in the gland, these dose rates give total doses of 82 rads per mci and 21 rads per mci to the respective tissues. The latter include parathyroid and perilaryngeal tissue.

Doses to Blood.

The dose to blood is also largely attributable to the 23.5 KeV per disintegration from electrons and from x-rays of energy less than approximately 4 KeV. The total blood dose can be estimated either by adding the contributions due to the iodide and thyroxine phases of iodine-125 or by the more fundamental approach developed by Gillespie and Orr (1969) and based on the occupancy principle. In this approach, which can be applied to a steady-state biological system through which there is a constant flow of mother substance, the total dose $D_{\beta i}$ delivered locally to a tissue i by a relatively long-lived electron-emitting isotope introduced into the entry to the system is given by:-

$$D_{\beta i} = 51.2 \, \bar{E}_{\beta} \frac{Q}{F} \frac{m_i}{M} \quad \text{rad} \quad \ldots\ldots\ldots\ldots \quad (1)$$

where $\bar{E}_{\beta}$ (MeV) is the local energy deposition/disintegration,

Q uci is the administered dose,

F (gm/day) is the flow of mother substance through the system and

$\frac{m_i}{M}$ $\left(\frac{\text{gm mother substance}}{\text{gm tissue}}\right)$ is the tissue content of mother substance.

Riggs (1952) has estimated that in the typical thyrotoxic subject the daily intake of iodine is 250 ug and the total iodine content of blood is approximately 84 ug per 1000 grams blood.

Substituting these values together with Q = 1 mci and $\bar{E}_\beta$ = 23.5 KeV in equation (1) gives the total dose to blood to be 0.41 rads per mci administered. Thus the radiation dose to blood from a therapeutic dose of iodine-125 is of the order of 0.4 rads per mci administered. An equivalent therapeutic dose of iodine-131 would give about 2.5 rads per mci administered (Green, Fisher, Miller and Wilson 1961). Thus ~~since~~ in the definitive trials now under consideration and in which it is proposed to use iodine-125 in doses of 10 - 25 mci the blood doses will not be higher than those currently incurred during conventional iodine-131 therapy.

Iodine-125 Therapy - Summary of Present Position.

When iodine-125 therapy is compared to iodine-131 therapy which is the only realistic comparison, the position can be summarised as in Figs F-12, F-13 and Table F-12. As far as can be determined from limited experience the advantages of a simple "out-patient single drink treatment" should be retained provided excessively detailed *in vivo* studies are not required before the dose is calculated. Certainly it would appear that iodine-125 given empirically in doses of the order of 400 - 600 uci per gram to the gland will control thyrotoxicosis and this might well be due to the relatively high doses given to the follicular cell apices. Compared to iodine-131, the iodine-125 doses to the follicular cell nuclei are relatively small, and the dose to the interfollicular stroma is lowest in the gland (Figs. F-12 and F-13). So far, however, these advantages remain hypothetical since the long term hypothyroid rate after effective iodine-125 therapy is not yet known. The

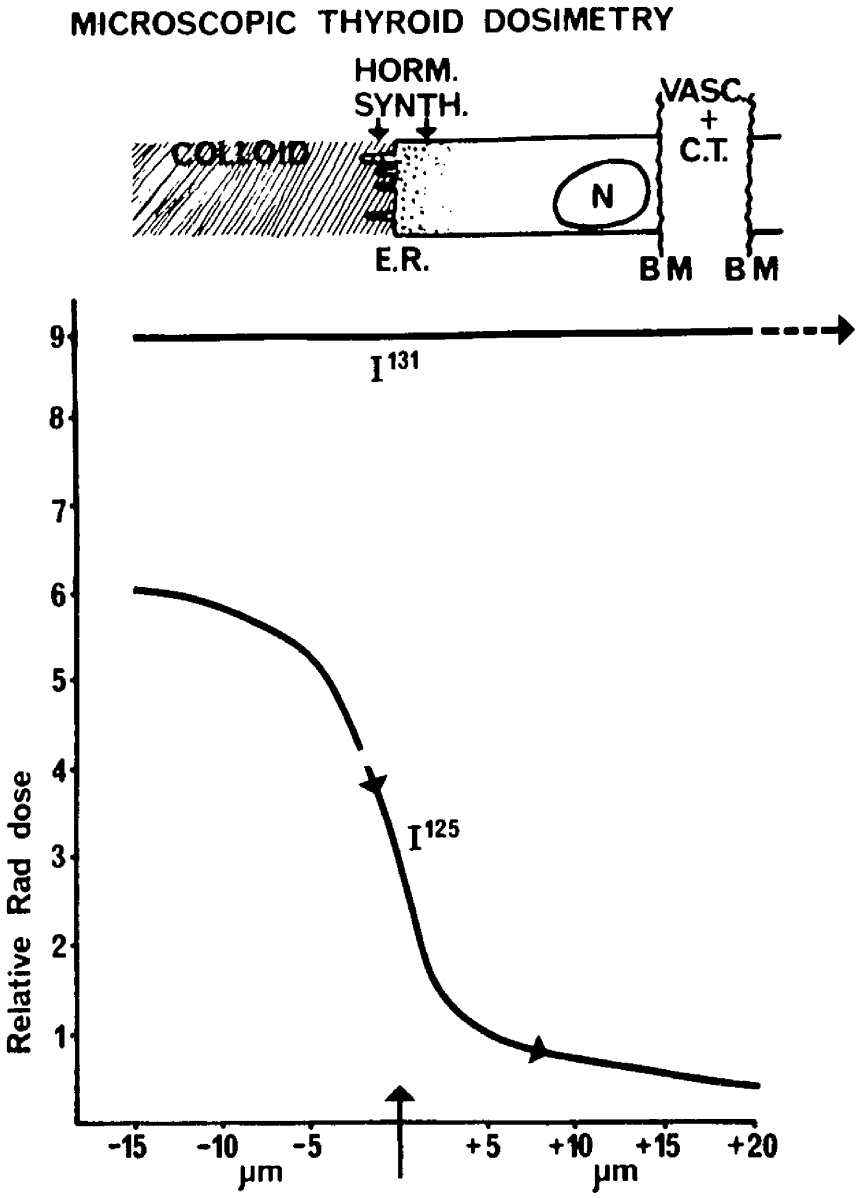


Fig. F-12 Summary of microdosimetry of iodine-125 and iodine-131 in thyrotoxic gland follicle. E.R. = endoplasmic reticulum. B.M. = basement membrane. Doses are from equal concentrations.

I_{125} IRRADIATION IN HUMAN THYROID. [Diagramatic]

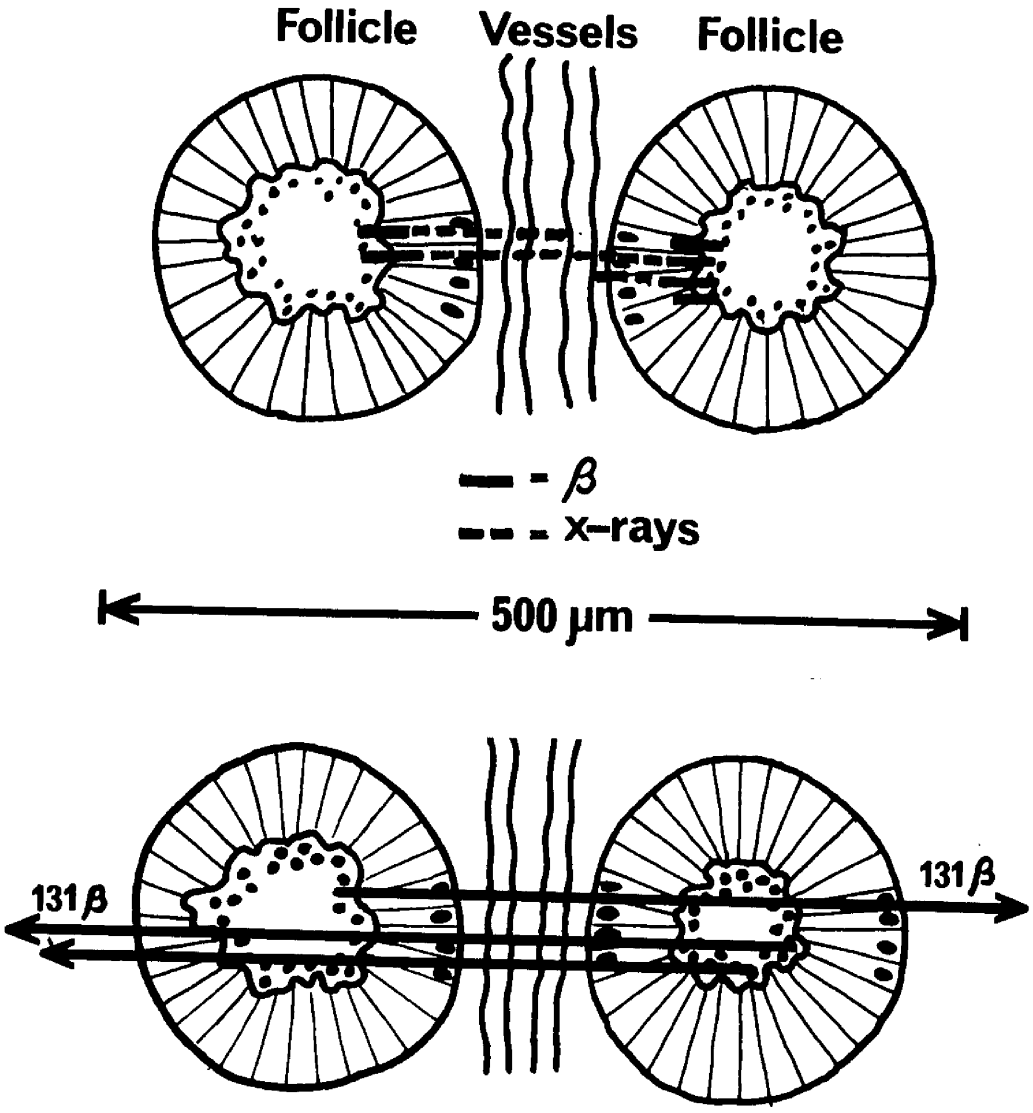


Fig. F-13 Diagrammatic view of microscopic dose distribution within and between contiguous thyrotoxic follicles. Note iodine-125 dose is inhomogeneous, with lower doses to nuclei and stroma.

fact that hypothyroidism was noted in 25 per cent of patients treated with 1000 uci iodine-125 per gram and after a follow-up of less than a year is not discouraging. These doses are unnecessarily high as witnessed by the rapid control rates. Furthermore, the occurrence of hypothyroidism in 15 per cent of the patients given 400 uc iodine-125 per gram thyroid, is not inconsistent with the belief that this could be eliminated by more appropriate dosimetry taking into account special factors such as "colloid mass".

In addition, since the mechanisms underlying delayed hypothyroidism after iodine-131 therapy appear to be linked to a combination of homogeneously high doses to all follicular cell nuclei and possibly to vascular injury too, and these are not characteristic of iodine-125 therapy, there may be very little delayed hypothyroidism after the latter irrespective of a small percentage of early hypothyroidism. In addition the calcitonin cells may be spared (Smith and Laljee 1967). These speculations can, however, only be examined by proper prospective clinical trials. These will also show whether thyrotoxicosis recurs after effective iodine-125 therapy; the recurrence rate after effective iodine-131 therapy is very low (1 per cent). Long term trials should also determine whether the risk of thyroid cancer, parathyroid injury (Harden, Harrison and Alexander 1963) laryngeal and post-cricoid cancer, leukaemia and genetic injury are as clinically negligible as they are after 20 years experience with iodine-131 therapy. The iodine-125 doses to the thyroid cells

TABLE F-12

Iodine-125 Therapy Compared to Iodine-131 Therapy - Summary

1. Advantage of simplicity to patient and physician.

2. Microscopic dose not homogeneous - dose to inner cell more than to cell nucleus and thyrotoxicosis is controllable.

3. Macroscopic dose not homogeneous - dose to one follicle may be greater than to another -- cell survival statistically more likely.

4. Dose to interfollicular stroma is lowest in gland and therefore vascular supply not impaired.

but with these theoretical advantages

1. Is hypothyroidism avoided in practice?

2. Is recurrence rate low?

3. Is thyroid cancer not a problem?

4. Is damage to parathyroids and other extrathyroidal tissues in neck not serious?

5. Is leukaemia or genetic damage negligible?

are not homogeneous and may be more carcinogenic for this reason. The doses incurred by tissues in the neck near or outside the thyroid are due to the low energy of photon radiation and are higher with iodine-125 therapy than they are with iodine-131 therapy. Thus parathyroid injury and post-cricoid cancer might arise. The doses incurred by the blood tissues ~~are~~ AND the ovaries are lower with effective iodine-125 therapy than they are with iodine-131 therapy but they are chiefly due to electronic irradiation much of which has energies below 10 KeV. At these low energies the R.B.E. in respect of the type of biological damage which leads to leukaemia and genetic damage is just not known and lymphocyte chromosome studies are unlikely to be prognostically helpful (Speight, Smith, Beba and Wilson 1968).

These considerations underly my conviction that long term trials of iodine-125 therapy should be most carefully conducted on patients over 40 years of age and only in a few specialised centres nominated in the U.K. by the Isotope Advisory Panel of the Medical Research Council.

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