

## Measurement of Radiation Effects on Thyroid Cell DNA Synthesis using Tritiated Thymidine

William R. Greig

*University Department of Medicine and Nuclear Medicine,  
Royal Infirmary, Glasgow, C.4., Scotland*

### 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% of the cells are follicular and about 30% are stromal.<sup>1</sup> 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.<sup>2</sup>

Ionising irradiations impair the capacity of the rat thyroid to undergo its normal 2 to 3 fold increase in weight in response to a continuous 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.<sup>3,5</sup> 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 on the follicular and stromal cells have been distinguished 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, RNA and DNA 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* and DNA synthesis too. 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 by Crooks *et al.*<sup>6</sup> The conditions were 300 KeV, 20 mA, 23 cm FSD, and the dose rate was 190 rad per 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 weighted 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  $\mu$ ) of thyroid were prepared (in a research pathology laboratory) from one lobe and viewed through a squared eyepiece 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 RNA and DNA.** The other thyroid lobe was stored at  $-20^{\circ}\text{C}$  and the total DNA and RNA estimated exactly as published by Begg, McGirr and Munro.<sup>7</sup> These methods are based on perchloric acid extraction and alkali digestion; DNA was measured by the Ceriotti colour reaction and RNA by optical density at 260  $\mu$ . Mean total DNA per thyroid and mean total RNA per thyroid for the rats in each group were expressed in  $\mu\text{g}$ . The DNA control was calfthymus DNA (Sigma/type 1), and RNA control was purified yeast (Sigma/type X1).

**DNA Synthesis.** When DNA synthesis was to be studied in a pulse label of tritiated thymidine (Thymidine-6-<sup>3</sup>H Radiochemical Centre, Amersham) was given intraperitoneally in a single dose of 0.5  $\mu\text{Ci}$  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.<sup>7</sup> The precipitate containing the DNA was digested using 0.6 N Nuclear Chicago Solubiliser in toluene.<sup>8, 9</sup> Counting was carried out in the Toluene-PPO-POPOP liquid scintillation system<sup>10</sup> and ultimately expressed as disintegrations per minute (d.p.m.) 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 DNA was not measured simultaneously with tritiated thymidine since it was found in preliminary experiments that the complete chemical procedure resulted in loss of label occurring at stage of alkali digestion.

**DNA labelling index.** Nuclear emulsion autoradiographs were also prepared using Kodak N.T.B. 2 nuclear emulsion exactly as described by Kopriwa and Leblond.<sup>11</sup> They were exposed at  $4^{\circ}\text{C}$  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 5000 cells. In all cases the labelled cells were randomly distributed throughout the tissue sections.

## EXPERIMENTS, RESULTS AND INTERPRETATIONS

### Experiment 1: Effects of X-rays on normal thyroid.

Thyroid weight, cell density, chemical DNA and RNA, tritiated thymidine incorporation and labelling index were determined at regular intervals before and after a single dose of 500 rad, a large dose in radiobiological terms. Groups of six rats were sacrificed at each of the nine times indicated.

The data (Fig. 1) demonstrate that in normal adult rats there is virtually no cell

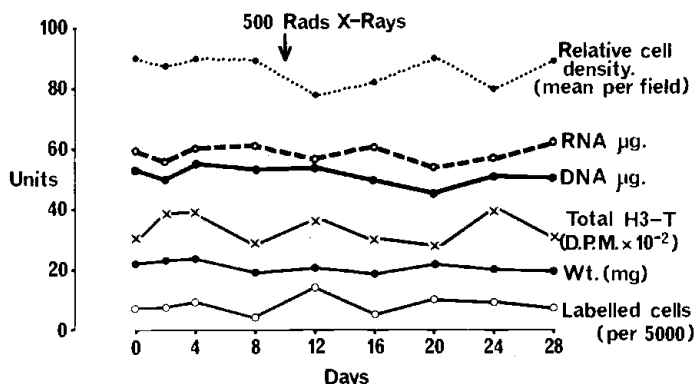


Fig. 1: Cell proliferation and DNA synthesis in normal rat thyroid. Sequential measurements on normal rat thyroid before and after single dose of X-rays (500 rad). Values are means from 6 animals per sacrifice.

proliferation and that a single X-ray dose of 500 rad 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  $G_1^2$  and that X-irradiation at a dose of 500 rad does not produce observable change. The next experiments describe the detailed changes in cell proliferation and DNA synthesis brought about by a goitrogenic challenge *without irradiation*; these provide control data for reference when the thyroids were X-irradiated then cell proliferation was promoted by the goitrogenic challenge.

### Experiment 2: Effects of a goitrogenic challenge on unirradiated thyroid.

Thyroid growth was artificially stimulated by providing 0.1% aqueous methyl-thiouracil (4-methyl-2-thiouracil, BDH Labs.) as drinking water and a diet of low iodine content (Nutritional Biochemical). Groups of five rats were sacrificed at each of the seven times indicated in Fig. 2, during which intervals the drug interrupts hormone genesis and the gland is stimulated by TSH.<sup>1 2</sup>

The data (Fig. 2) 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 rat thyroid cells were in generative cycle. Within two days of

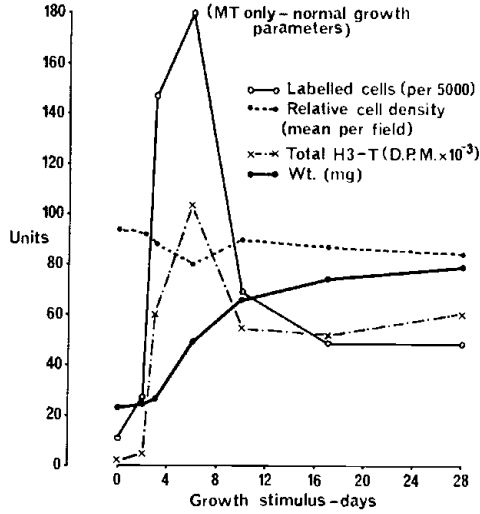


Fig. 2: Cell proliferation and DNA synthesis during goitrogenic challenge. No irradiation. Sequential measurements on rat thyroid before (0 days) and at intervals during continuous promotion of thyroid growth using 0.1 per cent methylthiouracil and low iodine diet for 28 days (goitrogenic challenge). Values are means from 5 animals per sacrifice. To be compared with Fig. 1.

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 eighth day and finally fell as growth slowed. Cell density dropped toward a small dip about day six followed by a slow fall, but the total decline was not substantial.

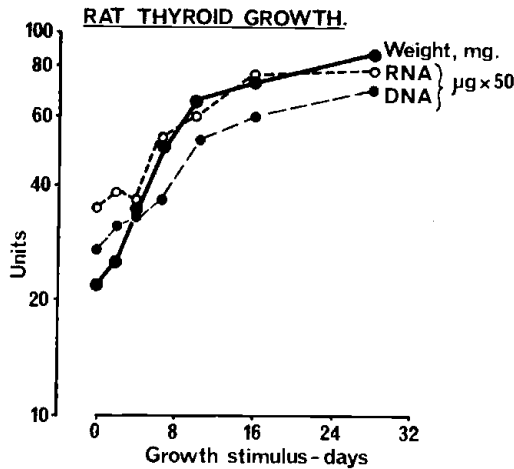


Fig. 3: Cell proliferation and RNA/DNA synthesis during goitrogenic challenge. No irradiation. Data are mean thyroid weight and chemical RNA/DNA—5 animals per group.

### Experiment 3.

In this experiment thyroid weight and chemical DNA and RNA were measured before and during a goitrogenic challenge. Seven groups of five rats were used. It should be noted that the ordinate scale in Fig. 3 is logarithmic. The graphs showed that DNA and RNA both rose in parallel with thyroid weight, but not quite to the same extent.

### Experiment 4.

Groups of three 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 measured. Figure 4 shows that the proportion of stromal cells increased from 30% to 42%.

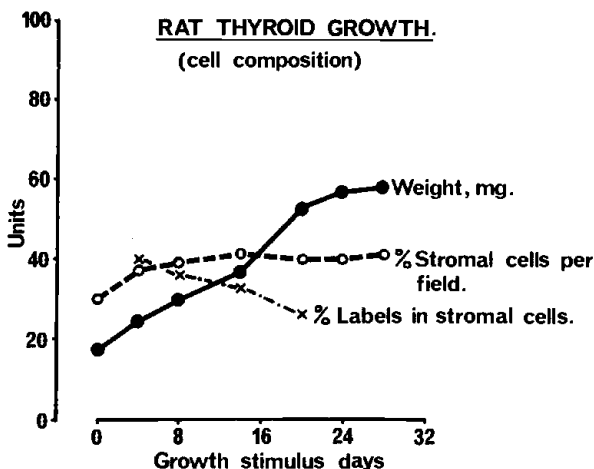


Fig. 4: Proliferation of stromal and follicular cells during goitrogenic challenge. No irradiation. Values are cells as percentage of total based on cell counts on 50 fields from 3 rats per sacrifice; per cent stromal cells is percentage of *all* cells; per cent labels in stromal cells is percentage of *all labelled* cells.

### Experiment 5.

In this experiment groups of five rats were commenced at day 0 on the goitrogen which was continued for five days. From the 5th to the 12th day, however, the goitrogen was removed and the rats took water and standard diet only. On 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. 5) 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 seven 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.<sup>13</sup>

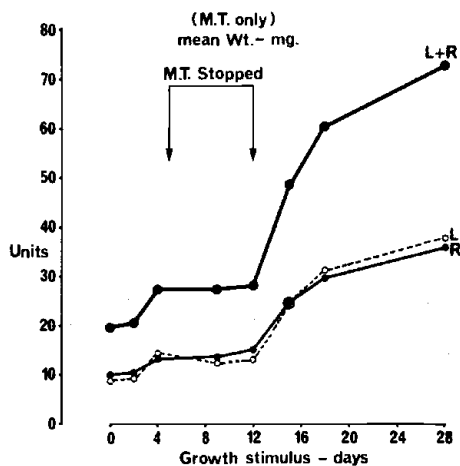


Fig. 5: Thyroid weight change pattern during temporary cessation of goitrogenic challenge. No irradiation. Goitrogen stopped from day 5 to day 12. Values are means from 5 animals per sacrifice.

### 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 (Figs. 1 and 2). The high peaks in both of these indices during goitrogenic stimulation (Figs. 2 and 3) corresponded to the maximum rate of increase of both weight and chemical DNA. The low labelling index found as the growth curve flattens off showed that the number of cells synthesising DNA simultaneously was much reduced as the growth slowed. The near parallel increases in chemical DNA and RNA and mass (Fig. 3) 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. 2. These conclusions were also reached recently by Philp *et al.*,<sup>5</sup> and, in general, by Doniach.<sup>14</sup>

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 DNA 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 DNA 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 deficiencies 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.<sup>13</sup> The evidence of experiment 4 showing a greater proportional rise in stromal cells (Fig. 4) 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 cells. The average contribution made by cell hypertrophy would appear to be the 20% which the total DNA, an index of cell number, failed, to rise proportionately to the total weight (Fig. 3). The proportional contribution to thyroid mass made by non-cellular structure other than colloid during goitrogenic growth is small.<sup>1, 15</sup>

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.<sup>16</sup>

### Experiment 6: Effects of a goitrogenic challenge on irradiated thyroid.

Single X-ray doses of 100 rad, 500 rad and 1000 rad were given to groups of rats. All animals were commenced on the goitrogenic regime 4 weeks after irradiation. Sub-groups of five animals from each X-ray dose group were sacrificed at the times indicated in Fig. 6, 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 responses to the goitrogen as revealed by thyroid weight, cell density, total tritiated thymidine incorporation, labelling index and stromal cell percentage, are shown in Figs. 6 to 10 respectively. Each figure is discussed separately and includes for comparative reference the data from experiments 2, 3 and 4 (Figs. 1, 2, 3 and 4), in which non-irradiated thyroids were subjected to the same goitrogenic procedure (control).

### Effect of X-irradiation on thyroid weight.

The greatest effect was seen after 1000 rad (Fig. 6). With this dose the thyroid

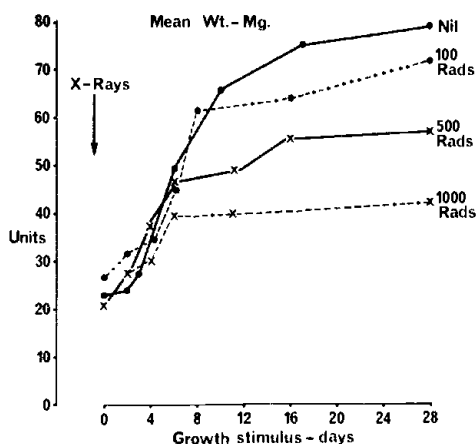


Fig. 6: Effects of X-irradiation on thyroid growth during goitrogenic growth stimulus, values are mean thyroids weights from 5 rats.

weight increased at a normal rate for nearly six days but then growth stopped. With 500 rad weight increased normally for six days and after six days growth was slow but not halted. 100 rad 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 rad and 1000 rad 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.

#### Lack of effect of X-irradiation on cell density.

There was no significant difference (Fig. 7) 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.

#### Effects of X-irradiation on stromal cell percentage.

The increase in stromal cells (percentage of total cells) during goitrogenic challenge was greater after 500 rad and 1000 rad than after 100 rad (Fig. 8) or no irradiation. This

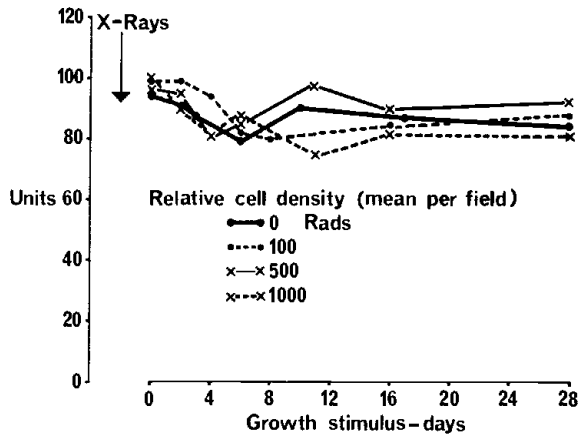


Fig. 7: Effects of X-irradiation on total thyroid cell density during goitrogenic growth stimulus, values are mean cell density in 50 fields from 5 thyroids.

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

#### Effects of X-irradiation on total tritiated thymidine incorporation.

The pattern of total gland tritiated thymidine ( $T-^3H$ ) incorporation after a dose of 100 rad was not significantly different from that of the non-irradiated controls. (Fig. 9).

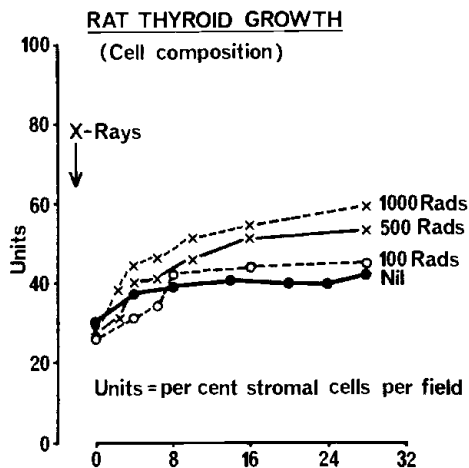


Fig. 8: Effects of X-irradiation on proliferation of stromal and follicular cells during goitrogenic growth stimulus. Values are per cent total cells based on cell counts in 50 fields from 5 rat thyroids.

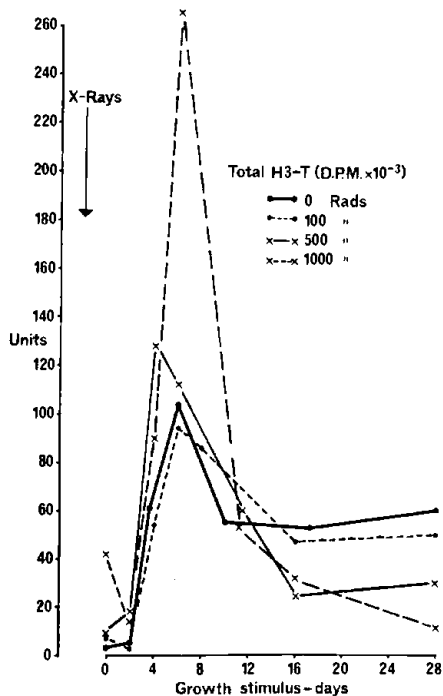


Fig. 9: Effects of X-irradiation on total thyroid incorporation of tritiated thymidine into DNA (total T-<sup>3</sup>H) during goitrogenic growth stimulus. Values are d.p.m. from 5 pooled thyroids.

Since, however, total T-<sup>3</sup>H uptakes, in the context of these investigations, are indices of average rates of DNA 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 rad showed a considerably higher peak in DNA 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. 6) and the 1000 rad peak was higher still.

These increased rates of DNA synthesis after both the higher X-ray doses must mean that the total amount of DNA 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 DNA synthesised at the last stages of growth (Fig. 9). Hence the total amounts of DNA 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 DNA synthesised in the first part of the goitrogenic response following 500 and 1000 rad could arise through three different mechanisms: more cells might be synthesising DNA at a normal rate or a normal number of cells could be synthesising DNA faster than normal, or fewer cells could be synthesising very much more DNA and rapidly. These possibilities were examined below in the light of the labelling indices data.

**Effects of X-irradiation on labelling index.**

In irradiated thyroid very large peaks in labelling index (labelled cells per 5000 cells) occurred at the same time as the unirradiated peak and after 1000 rad and 500 rad the labelling index was twice as high as in unirradiated gland (Fig. 10). The post-irradiation values reached 500 per 5000 cells compared to 250 for non-irradiated thyroid. These data are taken to mean that throughout the period of most vigorous cell DNA synthesis, twice

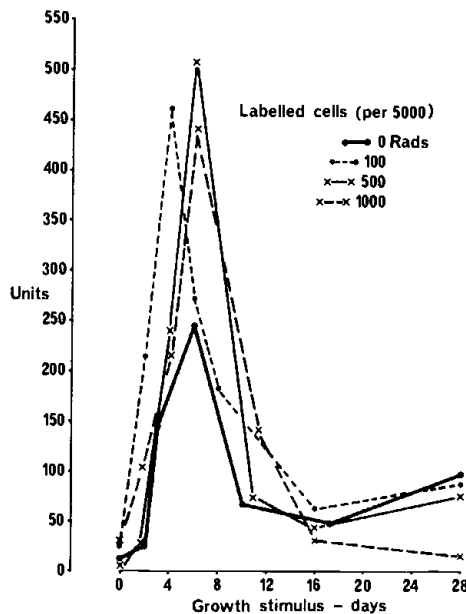


Fig. 10: Effects of X-irradiation on labelled thyroid cells (T-<sup>3</sup>H autoradiographs) per 5000 total cells during goitrogenic growth stimulus. Values are from 5 thyroids.

as many irradiated cells, as control cells, were in active DNA synthesis, and since they synthesised about twice as much total DNA as non-irradiated gland (Fig. 9) the mean rates must have been approximately normal. After this phase of high labelling (Fig. 10) the labelling indices fell, and after 1000 rad the fall was to below normal levels. Following 100 rad the labelling indices reached as high an abnormal peak as after 500 rad or 1000 rad. This contrasts with the relative normality of the total tritiated thymidine uptake (Fig. 9). Thus after 100 rad, although total DNA synthesis rates were normal, the number of cells involved during rapid thyroid growth were about doubled. A possible explanation for this is either that repair of DNA 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 rad; 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 DNA synthesis after 100 rad (Figs. 9 and 10) which did not significantly impair goitrogenic growth (Fig. 6), contrasted with the effects of 500 and 1000 rad which were decisive.

### **Comment on radiation effects on goitrogen induced thyroid growth**

The effects patterns which emerge with these high doses of X-irradiation and goitrogenic growth promotion over 28 days, are thus of an apparently normal initiation of growth and DNA 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 DNA. As a consequence, although growth is impaired or arrested at the phase of maximum potential increase (Fig. 6), the total uptake of tritiated thymidine and the proportion of cells labelled is higher than normal (Figs. 9 and 10). This interpretation is also consistent with the quick fall in total DNA synthesis, this probably showing that the number of cells involved after thyroid growth has ceased prematurely falls off sharply.

Other investigators<sup>17</sup> have incidentally noted increased uptake of tritiated thymidine after irradiating rat thyroid and giving iodine deficiency as a goitrogenic stimulus. Like them we think the increased cell DNA synthesis is the radio-biochemical counterpart of the large abnormal nuclei noted in morphological preparations of human thyroid following therapeutic doses of irradiation.<sup>18, 19</sup> These data show a high level of apparent abortive DNA 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*.<sup>20, 21</sup>

Increased DNA synthesis in other mammalian cells after their irradiation *in vivo* and during induced cycling has also been recently reported by Smets,<sup>22</sup> and Watanabe and Okada.<sup>23</sup>

### **Subsidiary experiment(s) 7.**

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 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.

In mice given tritiated thymidine intraperitoneally the retro-orbital blood tritium radioactivity time curve determined by the method of Hansen and Bush<sup>9</sup> was the same as

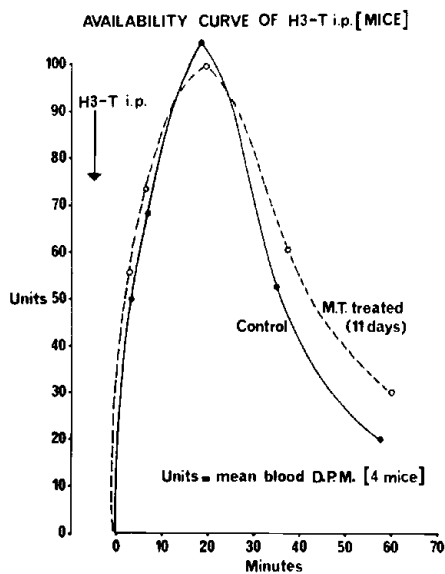


Fig. 11: Mouse blood tritiated thymidine (d.p.m.) curves after a single intraperitoneal dose ( $50 \mu\text{Ci}$ ). Comparison of curve in normals and methylthiouracil treated mice, values are means from same 4 mice.

that of untreated control mice (Fig. 11). In rats, the sequential changes in thyroid DNA 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. 12). Another experiment demonstrated that within the limits of specific activity used ( $17.0$  to  $28.0 \text{ Ci/mM}$ ), 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. 13). This appeared to exclude a possible pharmacological effect of the drug on the thyroid itself. These studies appear to prove that the changes in tritiated thymidine incorporation and labelling indices produced in the thyroid during the goitrogenic regime were not artefacts but reflected the real behaviour of the DNA 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 DNA and divide but at very low rates. The failure of a single dose of  $500 \text{ rad}$  of X-rays

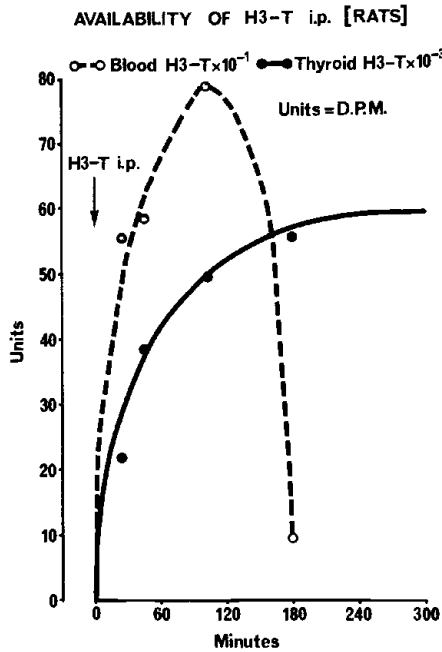


Fig. 12: Rat thyroid cell DNA tritiated thymidine (d.p.m.) and heart blood tritiated thymidine (d.p.m.) sequentially after a single intraperitoneal dose (0.5  $\mu$ Ci/gram). Values are means from 2 different pairs.

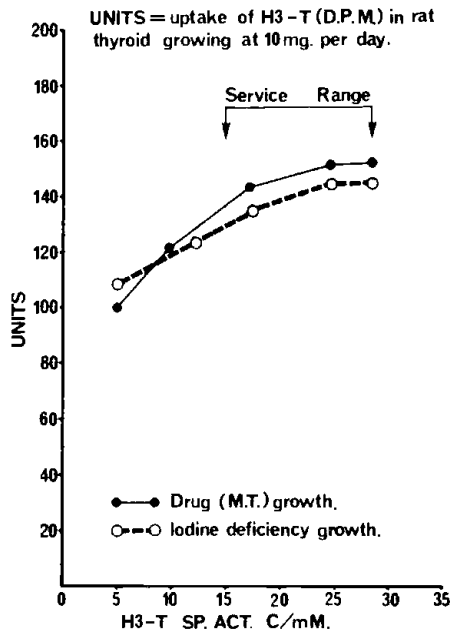


Fig. 13: Rat thyroid cell DNA tritiated thymidine (d.p.m.), 4 hours after single intraperitoneal dose (0.5  $\mu$ Ci/gram). Effects of varying the Sp. Activity; iodine deficiency growth compared with methyl thiouracil growth. Values are means from different pairs.

to perturb any of the parameters measured shows that any damage produced is not demonstrable using gland weight, cell density, chemical DNA or tritiated thymidine incorporation as the indices (Fig. 1). A single dose of 500 rad is a large dose by radiobiological standards producing gross disturbances in cell populations in active proliferation.<sup>24</sup> Since there is no migration of cells to and from the thyroid and the thyroid cell population is mainly in  $G_1$  or  $G_0$  damage produced by 500 rad 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. 2, 3 and 4 compared to Figs. 6 to 10 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. 2, 3 and 4). 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<sup>25</sup> 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 sub-compartments.

The irradiation effects (Figs. 6 to 10 inclusive) 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, correlated directly with X-ray dose (Fig. 6).

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.<sup>26</sup> Although the rates of DNA synthesis are severely affected by X-rays (Figs. 9 and 10) they do not alone form a simple quantitative index of radiation damage. Continued DNA synthesis without cell division and tissue growth makes interpretation of the radiation effect, if measured by DNA 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<sup>27</sup> so that there is a near constant relationship between weight, chemical DNA and RNA before and throughout the goitrogenic growth challenge (Figs. 1 and 3), The relatively steady index of cell density in the same conditions (Fig. 2) also suggests that increased cell number is the dominant change in goitrogen promoted thyroid growth. We estimate that the fraction of net thyroid growth not due to cell division is about 10%.<sup>28</sup>

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% 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 1200 rad).

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.<sup>3</sup> 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.<sup>9, 30, 31</sup> 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 studies 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.<sup>32</sup> 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.<sup>33</sup>

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## DISCUSSION

**L. Schutte:** Concerning discrimination between iodine-125 and tritium activity, did you consider combustion of your samples and subsequent chemical separation of iodine (e.g. as iodide) and water, after which you can count them separately?

**W. R. Greig:** Yes, this is one approach that we have in mind.

**C. P. Summers:** In practice in a liquid scintillation counter, it would not be possible to separate the  $\beta$  or electron emission with the purpose of distinguishing between iodine-125 and tritium spectra because the gamma emissions from iodine-125 would produce Compton electron, i.e. further  $\beta$  particles which would completely mask the  $\beta$  spectra. The best way of doing this measurement is to measure iodine-125 in a gamma counter and then iodine-125 and tritium in a liquid scintillation counter, having previously determined iodine-125-iodine-125/tritium counting ratio.

**W. R. Greig:** This is a valid point. I think the problem of counting small amounts of tritium in the presence of relatively large quantities of iodine-125 is not likely to be solved by the above approach; I suppose, however, that with careful calibration and appropriate controls the limit of this method could be defined, and should be.

**B. Legg:** Concerning the question of tritium and iodine-125 counting by liquid scintillation, surely a window can be selected above the tritium window to count the iodine-125,

the overlap in the tritium window determined, and the tritium count found by difference. Provided the tritium: iodine-125 ratio is above a certain value the result should be accurate. Alternatively, the sample can be counted and recounted after, say, one week when an appreciable amount of iodine-125 will have decayed. The iodine-125 can be calculated from the data and the tritium is again obtained by difference.

**W. R. Greig:** Certainly one would intuitively think that by multiple channel counting the tritium count could be distinguished from the iodine-125 counts but no-one has formally conducted this study and it is clear from this discussion that a project lies in this area. Recounting after a differential decay is difficult because during the latter the liquid scintillation system in the vial deteriorates.

**B. W. Fox:** Have you compared the tritiated thymidine incorporation following the damage from iodine-131 and iodine-125?

**W. R. Greig:** Not directly, but I have carried out autoradiographs of the labelled cell nuclei. Iodine-125 leaves more labelled cells than iodine-131 in equivalent rad doses (see W. R. Greig, PhD Thesis, University of Glasgow, 1970).

**J. A. B. Gibson:** What is the cell cycle time following 'treatment'? What evidence do you have *vis a vis* repair and an increase in the S phase which would increase the cycle time? It would obviously be useful to look at synchronised cells *in vitro* to see if S is increased or if there is take up during G1 or G2 indicative of repair.

**W. R. Greig:** I do not know the answers to these questions but I am pursuing the work using rat thyroid irradiated *in vivo*, cultured and labelled *in vitro*. Your comments are helpful.

**K. R. Harrap:** (i) Have you succeeded in demonstrating a differential effect against thyroxine synthesis in rat thyroid, comparing iodine-125 and iodine-131? (ii) Would you expect to eliminate thyroxine synthesis without impairing transcription of genetic information via prior irradiation of DNA?

**W. R. Greig:** (i) Using a variety of techniques I have attempted this and so far do not find that iodine-125 has a proportionally greater effect than iodine-131 on hormongenesis in rats but it might have in human thyrotoxicosis. (ii) On the basis of our current knowledge we might expect to impair the final stages of thyroxine synthesis and further studies are planned in this area.