Experimental induction of C cell tumours in thyroid by increased dietary content of vitamin D$_3$

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Abstract. Neonatal Wistar rats were given either 0, 5 or 10 $\mu$Ci $^{131}$I within 24 h of birth. Following weaning, they were fed a diet high, normal or deficient in vitamin D, for up to 2 years. Animals were sacrificed at approximately 3 monthly intervals, and serial sections of thyroid scanned for C cell tumours following calcitonin localization. Plasma calcium levels were also measured, and all results statistically analyzed. As expected, those animals given the high vitamin D had significantly raised calcium levels over those on a normal D diet, whilst those given a low D diet had lower calcium levels than normal. Analysis of the incidence of C cell tumours showed that those given a high D diet had significantly more C cell tumours, whilst those on a low D diet had significantly fewer than normal. Radiation dose also influenced C cell tumour incidence. There was a significant relationship between the vitamin D content of the diet and the incidence of C cell tumours, with those animals on a high D diet having the largest number of tumours. It is suggested that vitamin D or its metabolites may directly promote C cell growth, and that the high incidence of C cell tumours in the normal laboratory rat reflects the artificially high vitamin D content of the laboratory rat diet. The dietary vitamin D content may also be relevant to the variation in geographical incidence of medullary carcinoma in man. We consider it likely that vitamin D metabolites may play a significant role in the control of C cell function, hyperplasia and tumour formation in the rat, and that this may be more important in tumour formation than the role of serum calcium variation.

Although the C cells have been convincingly established as a second endocrine system within the thyroid gland and C cell tumours are common in the laboratory rat, little is known of the mechanisms controlling their growth and function. Whilst it is known that the C cells respond directly to raised serum calcium levels by the secretion of calcitonin (Copp 1970), previous work from this laboratory has shown that variations in dietary calcium did not significantly influence the incidence of C cell tumours, although serum calcium levels were significantly raised (Triggs & Williams 1977). These authors used a combination of physiological stress and radiation, known to cause tumours in other endocrine glands (Furth 1953) and in the follicular cells of the thyroid (Doniach 1958).

Excess dietary vitamin D$_2$ administration has been reported to produce C cell hypertrophy and hyperplasia in both cows (Young & Capen 1970) and in rats (Triggs & Bailey-Wood 1976). Several other studies have produced C cell hypertrophy and hyperplasia through administration of vitamin D$_3$ (Roszkiewicz 1974; Zabel 1976; Petkó 1979). It was therefore decided to examine the effect on C cell tumour incidence in rats of small doses of radiation followed by a variation in the dietary content of vitamin D$_3$ over a 2 year period.

Materials and Methods

**Animals**

During the first 24 h of life, Wistar Albino rats were injected ip, with either 0, 5 or 10 $\mu$Ci of carrier-free Na$^{131}$I. Each litter was adjusted to a size of 10 following injection of isotope, so that each neonate within one litter received the same radiation dose, thus avoiding problems due to the re-circulation of iodide within the litter (Doniach 1969).

At 24 days, 288 animals (144 males, 144 females) were...
weaned onto diets high, normal or deficient in vitamin D₃ to give a minimum of 96 in each of the three radiation groups, and a minimum of 32 in each of the three dietary subgroups. All animals were allowed to drink tap water ad libitum as it contained only 4.2 mg calcium/100 ml. Thirty-six animals (2 from each diet/sex/radiation group) were killed at 3, 6 and 12 months of age, and 45 animals at 3 monthly intervals from 15 to 24 months.

**Diet**

Vitamin D₃ was added to the basic diet which was composed of 30% extracted soya bean meal, 27.5% ground wheat, 15% wheat middlings, 10% ground barley, 5% maize gluten feed, 5% corn oil, and 5% molasses, and contained 0.68% phosphorus, 0.6% calcium and 2085 μg/kg iodide. Amino acid, mineral and vitamin supplements (excluding vitamin D₃) were added. The amount of vitamin D in the basic diet was not measurable, and this was used as the vitamin D deficient diet. It was supplemented with vitamin D₃ to give the amount normally present in laboratory rat diet (1000 IU/kg) and a high D diet (40 000 IU/kg). (Diets obtained from B. P. Nutrition UK Ltd., Stepfield, Witham, Essex, England).

**Experimental procedure**

During ether anaesthesia, rats were exsanguinated, and the thyroid excised immediately after death. Plasma calcium levels were measured by a modification of the method of Kessler & Wolfman (1964). One lobe of each thyroid was fixed in buffered formal saline for 24 to 36 h, and the other lobe fixed in glutaraldehyde picric acid (Solcia et al. 1968) for 6 h. Both lobes were dissected, weighed, processed and wax-embedded. The glands were serially sectioned at 5 μm, and five sections were mounted on each slide. Every third slice was stained with either H & E, PAS, or calcitonin, using an indirect antibody method described elsewhere (Thurston & Williams 1981). All sections from both lobes were scanned at a magnification of × 50, and the presence or absence of C cell tumours recorded. For the purposes of this study, a C cell tumour was regarded as a clearly demarcated mass of cells showing an abnormal growth pattern. Tumours of this morphology were regularly positive following calcitonin localization. Where an area of excessive C cell growth was seen which was not demarcated, it was regarded as hyperplasia.

**Results**

**Incidence of tumours**

C cell tumours were first seen at 15 months of age and showed a significant increase with age (P < 0.001). All results are therefore expressed in relation to animals aged between 15 and 24 months. A total of 19 C cell tumours were seen in this study, giving an overall incidence of 11% (19 out of 177). The sex incidence showed no significant difference, males showing 11 tumours (13%) and females 8 tumours (9%).

The distribution of tumours in different diet and radiation groups is shown in Table 1 and Fig. 1. Ideally the effect of diet should be considered first in the non-irradiated animals, and the effect of radiation in the normal diet animals. However, the numbers of tumours are too small for any firm conclusions.

<table>
<thead>
<tr>
<th>Radiation dose (μCi ¹³¹I)</th>
<th>Diet</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low D</td>
<td>Normal D</td>
</tr>
<tr>
<td>0 μCi</td>
<td>0/19</td>
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</tr>
<tr>
<td>5 μCi</td>
<td>1/20</td>
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<tr>
<td>10 μCi</td>
<td>1/20</td>
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</tr>
<tr>
<td>Total</td>
<td>2/59</td>
<td>6/59</td>
</tr>
</tbody>
</table>

**Fig. 1.**

Effect of radiation and dietary vitamin D content on the incidence of C cell tumours.

Table 1.

Numbers of C cell tumours in animals aged between 15 and 24 months.
When considering the combined effects of radiation and diet, two significant trends emerged. Firstly, the effect of radiation was examined in all diet groups (Table 1). There was no significant difference between the non-irradiated and 5 μCi groups, but the differences reached significance when comparing the 10 μCi with the 5 μCi group ($P < 0.05$). Secondly (Table 1 and Fig. 2) the effect of diet was examined in combined radiation groups. The differences in tumour incidence were significant ($P < 0.05$), showing a clear association with dietary content of vitamin D. The combination of factors which produced the greatest incidence of C cell tumours was 5 μCi $^{131}$I followed by a high D diet (Fig. 1).

**Calcium estimations**

The analysis of calcium levels was restricted to animals up to 12 months of age due to the subsequent appearance of C cell (and parathyroid) tumours. Means for diet groups were as follows: high D: $2.72 \pm 0.05$ mM, normal D: $2.51 \pm 0.03$ mM, low D: $2.46 \pm 0.03$ mM. Analysis of variance over this age range showed the effect of diet was highly significant ($F_{(2,46)} = 20.6$, $P < 0.001$). Individual Student's $t$-tests on the difference in calcium levels between diet groups showed that the highly significant effect was largely accounted for by the difference in calcium levels between the high and normal diet groups ($P < 0.001$). Although the low D calcium levels were lower than normal, this difference did not reach significance.

**Discussion**

These results show that both radiation and dietary vitamin D content significantly influence the incidence of C cell tumours in the rat thyroid. Although a large number of animals was used, the total number of C cell tumours was relatively small, and the results are therefore best discussed by merging all diet groups when discussing radiation effects, and by merging radiation groups when considering diet. A considerable number of follicular tumours was also produced in this experiment, so that all C cell tumours were classified using immunoperoxidase techniques for calcitonin.

Radiation led to an increase in the number of C cell tumours in the 5 μCi group of animals which was significantly greater than that in the 10 μCi group ($P < 0.05$) and although higher in the 5 μCi than in the 0 μCi animals, this difference did not reach significance. A previous similar experiment showed a significant increase in incidence of C cell tumours after irradiation (Triggs & Williams 1977). Our results are interpreted as being in agreement with this finding, with the additional feature that the 10 μCi dose in the current experiment led not only to a very great reduction in C cell number in the first few months after radiation, but also to a loss in regenerative capacity, presumably because of sterilization of the cells by this radiation dosage (Thurston & Williams 1981). Both radiation doses led to a reduction in C cell mass to less than 1% of normal at 3 months of age. The ability of these cells to regenerate was shown by the development of focal areas of C cell growth, which were much more obvious in the 5 μCi groups than the 10 μCi groups. If the tumour frequency were related to the post-radiation C cell mass, the importance of radiation carcinogenesis for C cells would be dramatically emphasized. This balance between carcinogenicity and cell destruction leads to a lower incidence of C cell tumours in the 10 μCi groups, and leads us to suggest that lower radiation doses may give a much higher incidence of C cell tumours.

The effect of dietary vitamin D on C cell tumour incidence was more dramatic than that of radia-
tion. There was a significant increase in tumour incidence with increasing amounts of dietary vitamin D ($P < 0.05$). This finding was not unexpected in view of the known effect of vitamin D in inducing C cell hyperplasia (Triggs & Bailey-Wood 1976), but it is important in relation to both the mechanisms that induce C cell hyperplasia and the possible factors concerned with the aetiology of C cell tumours in animals and in man. In a previous similar experiment, variation in dietary calcium was not associated with any significant change in incidence of C cell tumours (Triggs & Williams 1977). The changes in the serum calcium levels induced in the previous work are not directly comparable to those in the present work, but it is interesting that the low D diet used here was associated with a low incidence of C cell tumours in the absence of any significant changes in the serum calcium, while the low calcium diet used in the experiment of Triggs & Williams (1977) lowered the serum calcium levels, but did not lower the incidence of C cell tumours. This raises the possibility that vitamin D or one of its metabolites may be affecting C cell growth directly, rather than (or as well as) through the effect of vitamin D on calcium levels. It is therefore particularly interesting that vitamin D metabolites have been shown to influence the secretion of calcitonin directly. The metabolites predominant in the high D states, 24,25-dihydroxycholecalciferol (24,25-DHCC) and 25,26-DHCC, have both been shown to stimulate calcitonin secretion in vitamin D deficient pigs (Care et al. 1979), while 1,25-DHCC has been shown to reduce the secretion rate of calcitonin in pigs with an inherited form of pseudo vitamin D deficiency (Harmeyer et al. 1979). We therefore consider it likely that vitamin D metabolites may play a significant role in the control of C cell function, hyperplasia, and tumour formation in the rat, and that they may be more important in tumour formation than the role of serum calcium variation.

It is interesting to note that while the incidence of C cell tumours in the laboratory rat may be as high as 37% (Boorman et al. 1972) only one spontaneous thyroid tumour was found in 79 wild rats (Lindsay et al. 1968). It is difficult to induce rickets in the rat, even in severe vitamin D deprivation (Harrand & Hartles 1970), and it may well be that this species is adapted to a very low dietary vitamin D intake. It is therefore possible that the so-called high spontaneous incidence of C cell tumours in experimental strains of rat may be linked to a level of vitamin D in the laboratory diet that is very much higher than in the diet consumed in the wild. In addition the laboratory rat may well be exposed to much more daylight than the largely nocturnal wild animal.

In man there is probably a geographic variation in the incidence of medullary carcinoma (Williams et al. 1977). The importance of hyperplasia as a pre-neoplastic state in inherited medullary carcinoma in man is established (Wolfe et al. 1973), and dietary-induced hyperplasia precedes the onset of C cell tumours in the rat. It is therefore possible that dietary factors controlling C cell hyperplasia, such as vitamin D, may play a role in some cases of sporadic medullary carcinoma in man.

Acknowledgment

We are grateful to the Cancer Research Campaign for grant support.

References


Received on May 16th, 1981.