DNA synthesis and secretory activity in parathyroid adenomas

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Abstract. DNA synthesis was measured in vitro by incorporation of [3H]thymidine into DNA in 42 parathyroid chief cell adenomas immediately after surgical removal. The results ranged from 18 to 185 DPM/µg DNA and showed a positive correlation with pre-operative values for serum immunoreactive parathyroid hormone (r = + 0.47; P < 0.001) and plasma calcium (r = + 0.35; P < 0.05). There was no correlation between DNA synthesis and tumour weight or mean diameter of tumour cell nuclei. The results suggest that DNA synthesis and cell division in parathyroid adenomas are determined in part by the secretory activity of the tumour. DNA synthesis measured on one occasion is not necessarily an overall index of tumour growth.

Primary hyperparathyroidism exhibits a wide range of clinical and biochemical severity. One determinant of the expression of the disease may be the rate of growth of the parathyroid tumour (Lloyd 1968; Parfitt 1976). Investigation of the growth pattern of a parathyroid tumour throughout its existence is not possible, but information on growth rate at the time of operation can be obtained by measuring DNA synthesis in tumour tissue immediately after surgical removal. The present report describes the results of a study of 42 parathyroid adenomas showing that DNA synthesis was correlated with two indices of disease severity, serum immunoreactive parathyroid hormone (PTH) and plasma calcium. Data are also presented for the diameter of the nuclei of the parathyroid tumour cells, since this has recently been shown to be correlated with tumour weight and with disease severity (Lloyd et al. 1979).

Materials and Methods

To obtain a reasonably uniform sample of tumours, the study was restricted to parathyroid adenomas in which fat cells contributed less than 10% of the area of histological sections and in which at least 80% of the parathyroid cells were chief cells. In all cases, single adenomas proved to be responsible for the disease as judged by follow-up.

The age of the patients ranged from 19 to 86 years: 33 were female and 9 were male. Patients with renal insufficiency were excluded in order to minimize heterogeneity of circulating PTH due to altered excretion or renal metabolism. The series contained no cases of hyperparathyroidism with bone disease since no tumours from such cases met the histological criteria. There were 11 cases with kidney stones and 31 cases without kidney stones (or bone disease).

Serum PTH was measured by radioimmunoassay (Kleerekoper et al. 1974) using antisera prepared in the guinea pig to bovine PTH (AS 211/32, AS 211/41 Burroughs Wellcome). In our hands the intra-assay coefficient of variation was 9.5% and inter-assay 16%. Plasma calcium was measured by SMA (Technicon) analyser. Means of two to three pre-operative results for serum PTH and plasma calcium were used for computations. The parathyroid adenoma was handled as gently as possible during and after operation. It was weighed immediately after removal. DNA synthesis was estimated (Jacobi et al. 1977) in seven 2 to 3 mg pieces of each tumour free of blood and extraneous tissue. Within 30 to 45 min of surgical removal, the pieces were placed one to a flask in 2 ml medium 199 (calcium concentration 1.3 mM) at 37°C and gassed at 1 l/min with 95% O2 and 5% CO2. After 1 h pre-incubation, the medium was decanted and replaced with 2 ml medium 199 containing [3H]-
thymidine 1 μCi/ml (specific activity 20 Ci/mmol). Incubation was continued for 3 h and the pieces were then washed twice with 1 ml cold Tris-EDTA-borate buffer, pH 8.6, and homogenised by hand in the same buffer at 4°C. The homogenates were extracted for DNA, and DNA was estimated by the diphenylamine reaction. Radioactivity was measured in a liquid scintillation spectrometer. Incorporation of [3H]thymidine was found to be linear from 1 to 5 h incubation. In 19 adenomas, results (dpm/μg DNA) obtained with this method were compared with the nuclear label index after radioautography with [3H]thymidine and were closely correlated (r = +0.86; P < 0.001). Inter-assay variation was studied in pieces of rat liver set up with parathyroid experiments and was found to be negligible. Experiments with rat liver showed that leaving pieces of liver for up to 1 h at room temperature before commencing incubation did not affect DNA synthesis. The method has been validated in experiments on the rat pituitary gland stimulated by oestrogen, in which good agreement was obtained between results for mitotic index after colchicine arrest, DNA synthesis after in vivo injection of [3H]thymidine and the in vitro method described by Jacobi et al. (1977).

Histological examination of paraffin sections of each adenoma included assessment of cell types, fat content and content of non-cellular material, cysts and haemorrhagic areas. In addition, nuclear diameter was measured in 1000 cells (Lloyd et al. 1979) and mean nuclear diameter calculated. In tumours labelled in vitro with [3H]thymidine, the diameters of the labelled nuclei were measured.

Table 1.
Ranges of variables for parathyroid (chief cell) adenomas, serum PTH and plasma calcium (n = 42).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour weight (g)</td>
<td>0.12–6.95</td>
</tr>
<tr>
<td>Tumour DNA concentration (μg/mg)</td>
<td>2.20–9.94</td>
</tr>
<tr>
<td>Tumour DNA synthesis (dpm/μg DNA)</td>
<td>18–185</td>
</tr>
<tr>
<td>Tumour mean nuclear diameter (μm)</td>
<td>4.228–7.414</td>
</tr>
<tr>
<td>Serum PTH (ng/ml)</td>
<td>0.23–3.41</td>
</tr>
<tr>
<td>Plasma calcium (mmol/l)</td>
<td>2.50–3.58</td>
</tr>
</tbody>
</table>

Statistical treatment of results included calculation of correlation coefficients between pairs of variables after log transformation, using the SPSS computer programme.

Results

Tumour weight, serum PTH and plasma calcium occupied the wide range characteristic of this disease (Table 1). DNA concentration and DNA synthesis varied considerably between tumours (Table 1). For DNA concentrations, the mean of

![Fig. 1.](image)

*Fig. 1.*
Plot (log scale) of DNA synthesis (dpm/μg DNA) in parathyroid adenomas against serum PTH (ng/ml).

r = +0.47; n = 42; P < 0.001.
the intra-tumour standard errors was 13.7% (± SEM 1.3) of the tumour mean and for DNA synthesis 15.7% (± SEM 1.5). Significant correlations were found (Table 2) between tumour weight and serum PTH and plasma calcium, between DNA synthesis and serum PTH (Fig. 1) and plasma calcium, but not between DNA synthesis and tumour weight or total tumour DNA.

Mean nuclear diameter was correlated with tumour weight and with serum PTH, but not with DNA synthesis. In the tumours in which radioautography with [3H]thymidine was carried out, 76% of labelled nuclei were 6 μm in diameter, 16% 5 μm and 8% 7 μm.

Discussion

Although cell type and fat content were reasonably uniform throughout the sample of tumours, there were differences in DNA concentration and in mean nuclear diameter. DNA concentration was correlated inversely with tumour weight, but the range of DNA concentration was only partly explained by differences in cell size and the presence of extracellular material. Polyploidy, which is common in parathyroid adenomas (Bengtsson et al. 1977), might contribute to high DNA concentrations, but, although nuclear diameter has been shown to be correlated with the quantity of DNA in the nucleus (Bengtsson et al. 1977), we found no correlation between mean nuclear diameter and DNA concentration per mg tissue.

Validation of the method used to measure DNA synthesis is referred to above. In addition, the radioautographic study showed that 76% of the labelled (S-phase) nuclei were 6 μm in diameter. This is consistent with evidence that diploid parathyroid cells have nuclei of about 5.5 μm diameter and tetraploid about 7 μm (Lloyd et al. 1979), S-phase cells being in a state of transition.

The results for the serum PTH assay appear to be valid for the purpose of this study since they were correlated with plasma calcium, thus indicating their biological relevance. The significant correlation between tumour weight and plasma calcium or serum PTH confirms previous findings (Hodgkinson 1963; Purnell et al. 1974; Castleman & Roth 1978) and implies that most of the tumour cells are secreting and maintaining the plasma hormone level. Chief cells represent the secretory stage of parathyroid cells (Futrell et al. 1979) and made up the bulk of each tumour in the present sample. The correlation between serum PTH and DNA synthesis was independent of correlations between the latter and tumour weight or total tumour DNA and suggests that secretory activity going on throughout the tumour and reflected by the serum PTH level leads to or induces DNA synthesis in some of the cells regardless of tumour size. The correlation between plasma calcium and DNA synthesis raises another possibility since calcium influences DNA synthesis in normal parathyroid tissue (Lee & Roth 1975). However, the normal relationship is inverse in contrast to the present finding. If DNA synthesis in tumours were governed by a servomechanism reset to an abnormally high calcium, as has been suggested for the

Table 2.
Correlation coefficients (r values) between the pairs of variables indicated (n = 42).

<table>
<thead>
<tr>
<th></th>
<th>Tumour weight</th>
<th>DNA conc.</th>
<th>Total DNA</th>
<th>DNA synthesis</th>
<th>Plasma Ca</th>
<th>Serum PTH</th>
<th>Mean nuclear diameter (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour weight</td>
<td>+1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA conc.</td>
<td>-0.31*</td>
<td>+1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DNA</td>
<td>+0.93****</td>
<td>+0.07</td>
<td>+1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA synthesis</td>
<td>+0.20</td>
<td>-0.03</td>
<td>+0.20</td>
<td>+1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Ca</td>
<td>+0.55****</td>
<td>-0.10</td>
<td>+0.53***</td>
<td>+0.35*</td>
<td>+1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum PTH</td>
<td>+0.61****</td>
<td>-0.10</td>
<td>+0.61***</td>
<td>+0.47***</td>
<td>+0.56***</td>
<td>+1.00</td>
<td></td>
</tr>
<tr>
<td>Mean nuclear diameter (n = 37)</td>
<td>+0.41**</td>
<td>+0.07</td>
<td>+0.45**</td>
<td>+0.22</td>
<td>+0.21</td>
<td>+0.34*</td>
<td>+1.00</td>
</tr>
</tbody>
</table>

* P < 0.05.  ** P < 0.01.  *** P < 0.001.
secretory response (Murray et al. 1972), any correlation between DNA synthesis and plasma calcium should again be inverse.

From a previous study of nuclear diameters of parathyroid adenomas (Lloyd et al. 1979) it seemed possible that mean nuclear diameter reflected the rate of tumour growth. This is not supported by the present data which show no correlation between mean nuclear diameter and DNA synthesis.

Mechanisms relating DNA synthesis to secretory activity have not been established. In the prolactin cell, there is some evidence that lowering prolactin content may trigger DNA synthesis (Lloyd et al. 1978; Kalbermann et al. 1979) and raising it may inhibit DNA synthesis (Davies et al. 1974; Lloyd et al. 1975). Applied to the parathyroid cell (Parfit 1969), this idea has been challenged (Lee & Roth 1975) on the grounds that secretory granules increase in parathyroid tissue exposed to lowered concentrations of calcium which also stimulate DNA synthesis. However, the granules did not all contain PTH (Futrell et al. 1979). Moreover, adenoma cells may not able to store as much PTH as normal cells (Chertow et al. 1977) and adenomas differ in the suppressibility by calcium of their secretory activity (Brown et al. 1978). Hence an intracellular homeostatic role for PTH in relation to DNA synthesis deserves further investigation in normal and abnormal parathyroid tissue.

The relation of DNA synthesis measured on one occasion to the overall growth rate of a tumour is uncertain. Growth of a tumour depends upon new cell formation and the rate of cell loss, a factor not yet studied in the parathyroid to our knowledge. When hyperparathyroidism is observed to be increasing in severity, the present data suggest that, other things being equal, tumour DNA synthesis and/or tumour weight may be increasing. In early hyperparathyroidism, all adenomas may show low DNA synthesis. This may remain low in tumours causing chronic, mild disease or it may accelerate, giving rise to more severe disease. The present study does not reveal a separation of tumours into two types (Lloyd 1968), but includes no cases of hyperparathyroidism with osteitis fibrosa, which probably represents the most severe form of the disease.

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References


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