

## Nuclear diameter in parathyroid adenomas

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**SUMMARY** Nuclear diameter was measured in 55 parathyroid chief-cell adenomas to determine its value in histological diagnosis and to assess its relationship to other features of primary hyperparathyroidism. Mean nuclear diameter for the whole group of adenomas was significantly greater than that for the accompanying normal glands. Mean nuclear diameter in individual adenomas was significantly greater than that in the accompanying normal gland in 27 out of 34 cases. Nuclear diameter was correlated with tumour weight and with plasma calcium but was not correlated with duration of history. It was significantly greater in the group of patients with overt bone disease than in those with kidney stones and in those with neither kidney stones nor overt bone disease. Assessment of nuclear diameter is of value in histological diagnosis of parathyroid adenoma. The rate of growth of the adenoma may be a factor determining nuclear diameter.

In classic descriptions of the histology of parathyroid adenomas, attention is drawn to the size and appearance of the nuclei of the cells. According to Castleman (1952), the nucleus is 'usually large, filling about half the cell and quite hyperchromatic'. A variation in diameter of nuclei within the same tumour is also observed, some reaching as much as 20  $\mu\text{m}$ .

In a recent report, Bengtsson *et al.* (1977) described the frequent finding of polyploidy in parathyroid adenomas and made the observation that ploidity was correlated with nuclear diameter. In the present investigation, nuclear diameter was quantified in a series of parathyroid adenomas in order to determine its value in histological diagnosis and its significance as an index of growth and activity of the tumour.

### Material and methods

The material for investigation consisted of 55 parathyroid adenomas, the removal of which resulted in cure of the associated primary hyperparathyroidism. The study was restricted to adenomas in which at least 80% of the cells were chief cells. Biopsy at the time of operation provided portions of accompanying normal parathyroid glands in 34 of the patients.

Clinically, the cases fell into three groups: (1) overt bone disease defined by the presence of subperiosteal

erosions and/or cystic bone disease, (2) kidney stones but without overt bone disease, and (3) neither bone disease nor kidney stones. The presence of nephrocalcinosis was not a criterion of classification. Data for analysis included tumour weight, plasma calcium, age and sex of the patients, and, in a group of patients with kidney stones, duration of history.

The study also included five parathyroid glands removed at necropsy from patients without evidence of parathyroid disease or disorders of calcium metabolism.

The tissues were fixed in 10% formol saline and processed through 50%, 70%, and absolute ethanol and xylol. Paraffin sections of 4  $\mu\text{m}$  were stained with haematoxylin and eosin.

### DETERMINATION OF NUCLEAR DIAMETER

Nuclear diameter was estimated with the aid of a Wild ocular graticule, 0.2 mm square, divided into 100 squares. The graticule was calibrated with a stage micrometer for use on a Wild M-40 microscope. At  $\times 40$  magnification, one small square of the graticule was 4  $\mu\text{m}$  across. Nuclear diameters were measured by comparison with the 4  $\mu\text{m}$  squares. Diameters equal to or greater than 2  $\mu\text{m}$  were measured to the nearest 1  $\mu\text{m}$ . Fields were selected in which cells were arranged homogeneously, avoiding the edge of the section, distorted areas, and areas of haemorrhage. Endothelial cells were not included. In general, nuclei appeared round or slightly oval. In the case of oval nuclei, a mean diameter was

estimated. Elongated nuclei were avoided in case they represented artefacts of preparation. About 1000 nuclei were measured in each adenoma and sub-totals were recorded on a Ferrari-Statistest mechanical counter for each nuclear diameter from  $2\ \mu\text{m}$  to  $10\ \mu\text{m}$ . The number of fields required to include 1000 nuclei depended on cell size and density and ranged from three to eight. Nuclei greater than  $12\ \mu\text{m}$  in diameter were classified as giant nuclei. They were counted separately and not included in the calculation of mean nuclear diameter.

The measurements were carried out by the same observer throughout the study. Repeated assessments of individual adenomas were in very close agreement. In the same tumour, no significant regional differences were observed.

Mean nuclear diameter (MND) was calculated for each adenoma and for an accompanying normal gland. Significance of difference was calculated by Student's two-sample *t* test. Correlation coefficients (*r*) were calculated for log-values of the variables.

## Results

MND in the parathyroid adenomas ranged from  $4.228$  to  $7.907\ \mu\text{m}$  (mean  $5.831 \pm \text{SEM } 0.115$ ;  $n = 55$ ). In the accompanying normal glands, the range was  $4.394$  to  $5.571\ \mu\text{m}$  (mean  $4.970 \pm 0.052$ ;  $n = 34$ ). The group mean for adenomas was significantly greater ( $P < 0.001$ ) than that for the accompanying glands. In the necropsy specimens, the range of MND was  $4.800$  to  $5.390\ \mu\text{m}$  (mean  $5.032 \pm 0.104$ ;  $n = 5$ ) (Fig. 1).

The distribution of nuclear diameter in individual adenomas showed some variation but was approximately Gaussian (Fig. 2). Comparison of individual adenomas with their accompanying normal gland (Fig. 1) shows that MND of the adenoma was significantly greater in 27 cases, not significantly different in three, and significantly smaller in four. In the entire study, only one nucleus of  $2\ \mu\text{m}$  was seen. Nuclei of  $3\ \mu\text{m}$  were seen in 13/34 accompanying normal glands and in 13/55 adenomas (from types 2 and 3 cases only).

Excluding 'giant nuclei' ( $\geq 12\ \mu\text{m}$ ), the largest nuclei were  $9\ \mu\text{m}$  in diameter and were seen in 25 adenomas but in none of the accompanying normal glands.

Nuclei of  $8\ \mu\text{m}$  were seen in 43/55 of the adenomas and in 4/34 of the accompanying normal glands. Of these four glands, one had 8/1000  $8\ \mu\text{m}$  nuclei, one had 3/1000, and two had 1/1000.

Giant nuclei, ranging from  $12$  to  $20\ \mu\text{m}$ , appeared to fall outside the otherwise Gaussian distribution in a given adenoma. They were quantified as the number per 10 fields at  $\times 100$  magnification (about

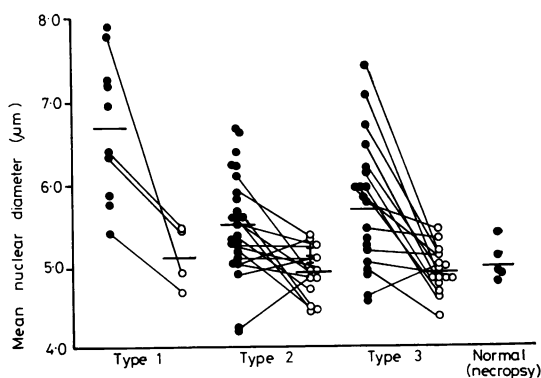


Fig. 1 Mean nuclear diameter ( $\mu\text{m}$ ) of individual parathyroid adenomas ( $\bullet$ ) and of one of their accompanying normal glands:  $\circ$  value significantly different from adenoma;  $+$  value not significantly different from adenoma. Horizontal bars indicate mean values. Type 1: patients with overt bone disease. Type 2: patients with kidney stones. Type 3: patients without overt bone disease or kidney stones. Normal glands: glands obtained at necropsy.

10 000 cells) and were found in 13/55 adenomas in a range of 1-27. No giant nuclei were found in accompanying normal glands.

## MND, TUMOUR WEIGHT, AND CLINICAL FEATURES

As a group, adenomas removed from patients with overt bone disease (type 1) showed a significantly greater MND than tumours associated with either type 2 ( $P < 0.001$ ) or type 3 ( $P < 0.01$ ) disease. However, the ranges overlapped considerably (Fig. 1). Mean tumour weight for type 1 cases ( $7.10\ \text{g} \pm 2.80$ ) was significantly greater ( $P < 0.01$ ) than that for type 2 cases ( $1.18\ \text{g} \pm 0.26$ ) and for type 3 cases ( $1.50\ \text{g} \pm 0.47$ ). For the entire group, MND showed no sex difference. An effect of age was observed in the female cases, tumours from patients under 50 years of age having slightly greater ( $P < 0.05$ ) MND ( $6.282\ \mu\text{m} \pm 0.352$ ;  $n = 11$ ) than from those over 50 years ( $5.661 \pm 0.123$ ;  $n = 34$ ).

Correlation coefficients between MND and tumour weight were significant for the whole group ( $r = +0.36$ ;  $P < 0.01$ ;  $n = 55$ ) but not for the three subgroups (types 1, 2, and 3). In 15 patients with a history of kidney stone ranging from 1 to 35 years, no significant correlation between MND and duration of history was found. There was a weakly significant negative correlation between MND and age of the patient ( $r = -0.32$ ;  $P < 0.05$ ;  $n = 54$ ).

MND was correlated with plasma calcium

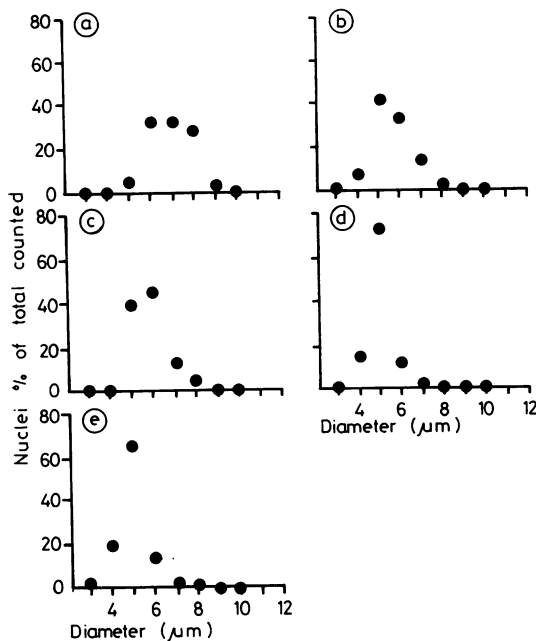


Fig. 2 Distribution of nuclear diameter ( $\mu\text{m}$ ) among approximately 1000 parathyroid cells in each case indicating typical findings for adenomas and normal glands, selected on the basis of mean value being closest to the mean value for the subgroup concerned. (a) Adenoma type 1, mean nuclear diameter =  $6.955 \pm 0.03$ ; (b) Adenoma type 2, mean nuclear diameter =  $5.560 \pm 0.03$ ; (c) Adenoma type 3, mean nuclear diameter =  $5.805 \pm 0.03$ ; (d) Accompanying normal gland, mean nuclear diameter =  $4.901 \pm 0.04$ ; (e) Normal gland (necropsy), mean nuclear diameter =  $4.915 \pm 0.03$ .

( $r = +0.43$ ;  $P < 0.01$ ) for the whole series but not for any of the three subgroups. Plasma calcium was correlated with tumour weight for the whole series ( $r = +0.54$ ;  $P < 0.001$ ), for type 2 cases ( $r = +0.46$ ;  $P < 0.05$ ) and for type 3 cases ( $r = +0.51$ ;  $P < 0.05$ ) but not for type 1 cases.

## Discussion

The results of this investigation support earlier observations concerning nuclear size in parathyroid adenomas (Castleman, 1952) and provide information of practical value in histological diagnosis. Nuclei of  $9 \mu\text{m}$  diameter were seen in about half of the adenomas but in none of the accompanying normal glands. The presence of nuclei of  $8 \mu\text{m}$  diameter was noted in all the adenomas but the accompanying normal glands contained nuclei of this size in only four cases. Mean nuclear diameter

was significantly higher in the adenoma than in its accompanying normal gland in 27 of the 34 cases in which both were available. There were no distinguishing features in the remaining seven adenomas nor in the three of these with mean nuclear diameters that were significantly smaller than those of the accompanying glands. In particular, there was no reason to change the diagnosis from single adenoma to primary hyperplasia in these cases (see below). The diagnostic value of nuclear diameter was evident in small tumours of 300 mg or less in that it was significantly higher than that of the accompanying gland in six of the seven cases in this category.

Bengtsson *et al.* (1977) established a relationship between nuclear diameter and ploidy in their study of parathyroid adenomas. Tetraploidy corresponded to nuclear diameter 28% above normal and octaploidy to a 70% increase. Extrapolation from their results to ours suggests that diploid nuclei are about  $5.5 \mu\text{m}$  in diameter, tetraploid nuclei  $7 \mu\text{m}$ , and octaploid  $9 \mu\text{m}$ . In these terms, about 20% of our tumours demonstrated tetraploidy in about 5% of cells and a similar percentage in 30 to 50% of cells. The significance of nuclei smaller than  $5 \mu\text{m}$  in diameter remains to be determined. We did not include cases of primary and secondary hyperplasia in this study because of lack of numbers. Bengtsson *et al.* (1977) found almost no evidence of tetraploidy in their cases of hyperplasia and observed no differences between hyperplastic glands. The absence of a difference between glands removed from the same patient therefore raises the possibility of chief cell hyperplasia.

Polyplody is common in both normal and neoplastic tissue (Kiefer and Sandritter, 1976). Bengtsson *et al.* (1977) commented that the mechanism of tetraploidisation in parathyroid adenoma is unknown, and these authors observed no correlation between the frequency of tetraploid cells and tumour weight, serum calcium, or duration of symptoms. In the present investigation, mean nuclear diameter was correlated with tumour weight and with plasma calcium but not with duration of existence of the tumour, roughly indicated by the duration of the history. The differences in mean nuclear diameter between the clinical subgroups (types 1 and 2 and 1 and 3) may have been dependent partly on differences in tumour weight. However, it has been suggested that type 1 hyperparathyroidism represents severe progressive disease caused by tumours of relatively rapid growth (Lloyd, 1968). If this is the case, then increased nuclear diameter and, by inference, polyplody may be associated with rapid tumour growth. On the other hand, absence of polyplody in primary and secondary parathyroid hyperplasia (Bengtsson *et al.*, 1977) argues against

rapid growth as a cause of this phenomenon, the mechanism of which remains uncertain (Brodsky and Uryvaeva, 1977).

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