Intrafollicular amyloid in primary hyperparathyroidism

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SYNOPSIS The histology of the parathyroids from 88 cases of primary hyperparathyroidism has been reviewed in a search for local amyloid deposits.

Characteristic intrafollicular amyloid deposits of varying extent were found in nine cases. The case histories of these show that seven had suspected or proven pluriglandular adenomatosis but that the remainder had no such associations. The material studied shows no correlation with systemic primary or secondary amyloidosis.

The significance of these findings is discussed in relation to the pluriglandular syndrome, peptide hormones, medullary carcinoma of the thyroid, and calcitonin secretion. It is suggested that amyloid in this context may be a ‘marker’ for secretion of a peptide closely related to calcitonin.

Local deposition of amyloid in tumours of endocrine glands is well documented in medullary carcinoma of the thyroid and islet cell tumours of the pancreas (Porta, Yerry, and Scott, 1962; Williams, Brown, and Doniach, 1966). The finding of fairly large amounts of amyloid in a parathyroid removed for primary hyperparathyroidism and showing chief cell hyperplasia prompted a search for further cases.

Methods

Material from 88 patients was examined; 66 were from the London Hospital of whom 64 were cases of surgically treated primary hyperparathyroidism and two were necropsies to which our attention was drawn during the survey. The remaining 22 patients had parathyroids removed for primary hyperparathyroidism at King’s College Hospital. Forty cases showed chief cell adenomas and 48 primary hyperplasia (Table I).

Haematoxylin-and-eosin-stained sections were screened for deposits of amorphous eosinophilic material resembling amyloid, and the development of anomalous colours when viewed between crossed polaroids (Brewer, 1964) was used as an additional check to eliminate the colloid often seen in parathyroids with a follicular pattern. Amyloid was then more definitely identified by Highman’s Congo red and Thioflavine T stains. Methyl violet stains were used but were not consistent, being unhelpful in doubtful cases. Known primary and secondary amyloid sections were used as controls on all occasions. Examination of Congo red sections between crossed polaroids for dichroism (Ladewig, 1945) proved most useful; this method is regarded by most authorities as the best histological method for identifying amyloid (Cohen, 1967). Cases not showing dichroism were excluded. It was possible in one case to reprocess material from a paraffin block and examine it using the electron microscope.

Results

A follicular pattern is common in hyperplastic parathyroids and the follicles often contain
amorphous colloid. In the present series intrafollicular amyloid deposits were detected in nine of the 88 cases examined; seven of these nine cases had suspected or proven pluriglandular adenomatosis and two had no such association (Table I).

The main pathological findings in the parathyroids are summarized in Table II and the clinical and other associated features in Table III.

In the nine positive cases there is a wide age range (24-61 yr) and almost equal sex distribution (five female, four male). In seven cases the glands showed chief cell hyperplasia and in the remaining two there were chief cell adenomas. The largest amyloid deposits were seen in the first parathyroid gland removed from case 1 (Table II); follicular areas showed extensive intrafollicular amyloid deposits (Fig. 1). The cells surrounding the amyloid deposits were of moderate size with a round nucleus towards the base and the cytoplasm contained clear vacuoles. The staining reactions of these cells by PAS, Best's carmine, Bodian, and diazo methods did not differ from those of the adjacent follicular and non-follicular parathyroid tissue not containing amyloid. In addition to these large and obvious areas of intrafollicular amyloid the adjacent hyperplastic parathyroid tissue contained similar small foci of amyloid with tiny follicles scattered throughout the gland (Fig. 2). It was these small deposits which were found in most of the other cases (Fig. 3), although in some (case 6) larger areas were seen (Fig. 4).

Table I Intrafollicular amyloid in parathyroids

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Date</th>
<th>Site</th>
<th>Amyloid</th>
<th>CR</th>
<th>TT</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49F</td>
<td>(a) 1956</td>
<td>RL</td>
<td>+</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24M</td>
<td>1956</td>
<td>RU</td>
<td>-</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>44F</td>
<td>P.M.1956</td>
<td>RL</td>
<td>-</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>60M</td>
<td>1951</td>
<td>LL</td>
<td>-</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>38F</td>
<td>(a) 1960</td>
<td>LL</td>
<td>-</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40M</td>
<td>P.M.1947</td>
<td>LL</td>
<td>-</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>49M</td>
<td>(a) 1964</td>
<td>LL</td>
<td>-</td>
<td>Chief cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>48F</td>
<td>1964</td>
<td>LU</td>
<td>-</td>
<td>Chief cell adenoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>61F</td>
<td>1962</td>
<td>LU</td>
<td>-</td>
<td>Chief cell adenoma</td>
<td></td>
<td></td>
<td></td>
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</table>

Table II Parathyroid histology

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Relationship</th>
<th>Age</th>
<th>Sex</th>
<th>Lesions</th>
<th>Bone Lesions</th>
<th>Gastrointestinal Tract</th>
<th>Other Relevant Features</th>
<th>Present Condition</th>
<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mother of case 2</td>
<td>49F</td>
<td>Nephrocalcinosis</td>
<td>calci</td>
<td>0</td>
<td>Recurrent duodenal ulcer</td>
<td>Islet cell tumour</td>
<td>Raised serum Ca$^{++}$</td>
<td>Nine siblings, 3 sisters (1 and 3) with DU and pluriglandular syndrome, 1 sister haematemesis, brother dead uraemic, and renal calculi, remainder alive and well. Uter history in two previous generations</td>
</tr>
<tr>
<td>2</td>
<td>Son of case 1</td>
<td>24M</td>
<td>None</td>
<td>0</td>
<td>Epigastric pain, high acidity</td>
<td>None</td>
<td>Died 1956 Necropsy</td>
<td>Well</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sister of case 1</td>
<td>44F</td>
<td>Calci; severe renal failure, nephrocalcinosis</td>
<td>Osteoporosis</td>
<td>Recurrent duodenal ulcer</td>
<td>Islet cell tumour, adrenal cortical adenoma</td>
<td>Died 1947 Necropsy</td>
<td>Only sister of 5, bronchoeal carcinoma; only sister 4, renal calculi and pituitary adenoma</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Father of case 5</td>
<td>60M</td>
<td>Calci</td>
<td>0</td>
<td>0</td>
<td>Lipoma</td>
<td>Died 1964 Raised serum Ca$^{++}$ when last seen. No necropsy</td>
<td>Well</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Daughter of case 4</td>
<td>38F</td>
<td>Nephrocalcinosis</td>
<td>0</td>
<td>0</td>
<td>Pituitary adenoma, lipoma</td>
<td>Died 1947 Necropsy</td>
<td>No family history discovered</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>40M</td>
<td>Adenoma</td>
<td>0</td>
<td>0</td>
<td>Lipomatosa, islet cell tumour, pituitary adenoma, adrenal cortical adenoma Argentaffinoma of small bowel</td>
<td>Died 1947 Necropsy</td>
<td>No necropsy. Carcinoma breast</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>49M</td>
<td>Nephrocalcinosis</td>
<td>Osteoporosis</td>
<td>Malabsorption, high gastric acidity</td>
<td>Islet cell tumour, pituitary adenoma, adrenal cortical adenoma Argentaffinoma of small bowel</td>
<td>Died 1969</td>
<td>No necropsy. Carcinoma breast</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>48F</td>
<td>Calci</td>
<td>Osteoelastic jaw lesion</td>
<td>0</td>
<td>0</td>
<td>Died 1969. No necropsy. Carcinoma breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>61F</td>
<td>Calci</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Died 1969. No necropsy. Carcinoma breast</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III Clinical features...
Intrafollicular amyloid in primary hyperparathyroidism

was often possible to examine more than one parathyroid from each case, and indeed cases 1, 5, and 7 had recurrent hyperparathyroidism necessitating further operations. It is apparent (Table II) that not all parathyroids from each case showed these deposits but this may merely represent a random distribution.

The deposits were not related to the site of the parathyroid. An impression was formed that they tend to occur near the periphery of the gland. Localized islands of these small amyloid-containing acini were not seen.

In addition to the 88 cases of primary hyperparathyroidism 20 normal parathyroids from

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Fig. 1 Case 1. Parathyroid showing extensive intrafollicular amyloid deposits. Concentric deposition is present and breakdown of follicular structure can be seen. Haematoxylin and eosin $\times$ 160.

Fig. 2 Case 1. Parathyroid with chief cell hyperplasia and small, sometimes laminated intrafollicular deposits of amyloid (arrows). Haematoxylin and eosin $\times$ 640.

Fig. 3 Case 9. Parathyroid adenoma showing small birefringent intrafollicular amyloid with a Maltese cross appearance. Congo red with crossed polaroids $\times$ 640.
routine postmortem cases were examined. Amyloid was not detected in any of these. Some corpora amylacea in prostate and lung were also examined. Those in the prostate took up Congo red but did not show birefringence. Some in the lung showed a regular laminated yellow-green birefringence but not the broken-up, apple-green dichroism as is seen in amyloid (Fig. 5).

**AMYLOID**

The amyloid present showed dichroism in sections stained with Congo red and viewed in a polarizing microscope, and green fluorescence with thioflavine T stains in all cases (Fig. 5). The tiny intrafollicular deposits often gave a Maltese cross pattern of birefringence (Fig. 3). Larger deposits sometimes had a concentric layered appearance (Figs. 1 and 5). In case 1 the amyloid had broken out of some follicles into the stroma (Fig. 1) and macrophages with a few foreign body giant cells were closely applied to it within some large, possibly confluent, follicles (Fig. 6). Amyloid was not seen involving other elements.

**Fig. 4** Case 6. Parathyroid with large birefringent intrafollicular amyloid deposits. Congo red with crossed polaroids × 160.

**Fig. 5** Case 1. Parathyroid showing the characteristic broken-up, green-yellow dichroism of amyloid. Lamination is present in some deposits. Congo red with crossed polaroids × 160.

**Fig. 6** Case 1. Parathyroid amyloid with macrophage and foreign body giant cell reaction. Haematoxylin and eosin × 160.

Electron microscopy of tissue from case 1 showed the non-branching fibrillar network composed of long, 75-100Å wide, fibres (Fig. 7) which, together with the staining properties previously referred to, characterize amyloid. Electron microscope controls of known amyloid material, also previously paraffin-embedded, appeared identical.
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When the clinical records of cases 1, 2, 4, and 5 were examined it was realized that they were members of two families having the pluriglandular syndrome, and that case 5 had been the subject of a previous report (Greenbaum, 1960). This led to the examination of the postmortem records of case 3 (sister of case 1) and case 6, the subject of a pluriglandular syndrome previously reported (Marshall and Sloper, 1954). Case 7 had had a small intestinal argentaffinoma removed and is known to have a high gastric acidity. There are therefore certainly six and probably seven cases of pluriglandular syndrome (Table III), five of these coming from two families (cases 1, 2, 3, 4, and 5). The parathyroids from other members of these families who had died or had resections were not available for study. Cases 8 and 9 had no such family or personal history.

With the exception of case 2 all cases show renal calcification of greater or lesser extent, in some progressing to severe renal failure. Only case 8 had a bone lesion unequivocally due to primary hyperparathyroidism. In case 3 there was longstanding renal disease and case 7 had intestinal malabsorption which might have produced bone changes seen radiologically. None of the three islet cell tumours (cases 1, 3, and 6) contained amyloid.

Discussion

Amyloid deposits are not mentioned in the many reviews of primary hyperplasia of the parathyroid gland (Woolner, Keating, and Black, 1952; Cope, Keynes, Roth, and Castleman, 1958; Black, 1960; Pyrah, Hodgkinson, and Anderson, 1966), familial hyperparathyroidism (Cutler, Reiss, and Ackerman, 1964; Schachner, Riley, Old, Taft, and Hamwi, 1966; Stevens, Bloomer, and Castleton, 1967) and pluriglandular adenomatosis (Underdahl, Woolner, and Black, 1953; Wermer, 1963; Ballard, Frame, and Hartsock, 1964). The reason for this lack of previous observation may reflect the fact that only tiny deposits are present in most glands. The significance of the observation is of greater interest. It must be emphasized that these deposits do not relate to systemic amyloidosis either primary, familial, secondary, or senile. A careful study of postmortem material and records of the patients and families failed to reveal any such associations. The cases presented show no predisposition to deposit amyloid anywhere other than in the parathyroid. The local amyloid must therefore be interpreted with reference to similar deposits in other endocrine-secreting tissue and to the normal or abnormal functions of the parathyroid glands.

The isolated amyloid fibril has been shown to be a protein (Cohen, 1966), and therefore largely composed of peptides. Local deposits of amyloid are known to occur in islet cell tumours of the pancreas (Porta et al, 1962), medullary carcinoma of the thyroid (Hazard, Hawk, and Crile, 1959; Williams et al, 1966), and are now shown in the parathyroid. Peptide hormones are produced at all these sites and it is possible that the amyloid may represent polymerization of an aberrant peptide which may or may not be related to the particular hormone. However, amyloid deposits have not been recorded in pituitary tumours, carcinoid tumours, or oat-cell carcinoma of the bronchus associated with endocrine syndromes, all of which are known to produce peptide hormones. Thus the relationship may be more specifically with the three sites mentioned.

Amyloid deposits are a prominent feature of medullary carcinoma of the thyroid but their significance has not been elucidated and is not referred to in reviews of the subject (Hazard et al, 1959; Williams et al, 1966). In these tumours the amyloid is stromal and not intrafollicular as in the parathyroids, where the appearances suggest...
a relationship to the secretions of surrounding cells. Small amyloid deposits completely surround-
ed by tumour cells are present in some medullary carcinomas (Williams et al, 1966). Tissue culture of
medullary carcinoma shows rosette formation by the tumour cells with amyloid at the centre
(Albores-Saavedra, Rose, Ibanez, Russell, Grey, and Dmochowski, 1964), and amyloid fibrils
have been seen in the cytoplasm of the tumour cells indicating production of amyloid by these
cells (Gonzalez-Licea, Hartmann, and Yardley, 1968; Meyer, 1968). The intrafollicular position of
the amyloid in the parathyroids has the same connotation, and it should be noted that it is only
in the larger follicles that macrophages can be seen and not in the tiny acini where amyloid is
presumably just beginning to be deposited.

It has been shown that medullary carcinoma secretes calcitonin (Tashjian and Melvin, 1968;
Cunliffe, Black, Hall, Johnston, Hudson, Shuster, Gudmundsson, Joplin, Williams, Woodhouse, Galante, and MacIntyre, 1968;
Tubiana, Milhaud, Coutis, Lacour, Parmentier, and Bok, 1968), a peptide hormone with hypo-
calcaemic and hypophosphataemic action due to inhibition of bone resorption (Copp, Cameron, 
Cheney, Davidson, and Henze, 1962; Friedmann and Raisz, 1965). Calcitonin-like activity has also
been identified in normal human plasma (Gudmundsson, Byfield, Galante, Kenny, MacIntyre, 
Osafo, and Tse, 1968), and it is probable that this hormone directly opposes parathormone in
its bone resorptive action. Calcitonin is secreted by C-cells (Pearse, 1966) derived from the last
branchial pouch, and medullary carcinoma of thyroid is almost certainly a tumour of these cells
(Williams, 1966). Amyloid deposition may be directly related to calcitonin secretion in medullary
carcinoma, possibly representing aggregates of a closely related polypeptide produced by altered
cellular metabolism, or even a normal calcitonin precursor produced in excess. Thus it may act as
a marker of calcitonin secretion, and it is suggested that the same may apply in the parathyroid.

Hypercalcaemia and calcitonin secretion are not incompatible as calcitonin only opposes parathormone in its actions on bone and not its effects in increasing calcium absorption from the intesti-
nal tract (Spencer, Lewin, and Samachson, 1968).

In support of the 'marker' hypothesis it should
be noted that calcitonin activity has been shown
in the human parathyroid, thyroid, and thymus
(Galante, Gudmundsson, Matthews, Tse, Williams, Woodhouse, and MacIntyre, 1968).
Thus it would appear that C-cells can be incorpo-
rated embryologically into all three glands. It
would also be more likely that a hyperplastic or 
adenomatous gland would produce peptides closely related to its normal metabolism, whereas
a variety of unrelated peptides might be produced
by a neoplastic cell.

Against this view it might be argued first that
intrafollicular amyloid should be seen in hyper-
parathyroidism much more frequently than
indicated by the present study since the stimulus
for calcitonin release is hypercalcaemia (Copp
et al, 1962; Kumar, Foster, and MacIntyre, 1963).
However, it is not known whether the normal
distribution of C-cells is uniform between para-
thyroid, thyroid, and thymus, and it is possible
that there is considerable individual variation: a
search for tiny deposits of amyloid within the
thyroid or thymus of hypercalcaemic patients
might be rewarding. Secondly there is our inabi-
ity in this material either to identify the folli-
cular cells as C-cells or to differentiate them
from the surrounding parathyroid cells not
associated with amyloid. C-cells are argyrophil
(Nonidez, 1932) but, since argyrophilia is also one
of the characteristics of normal parathyroid cells
(Roth, 1962), it is not a distinguishing character-
istic. More detailed identification of these folli-
cular cells must await the availability of material
suitable for electron microscopy allowing a search
for the secretory granules as seen in C-cells (Meyer
and Abdel-Bari, 1968) and for immunofluorescent
studies to locate the site of calcitonin production
in the parathyroid.

If amyloid deposition does relate to calcitonin
in medullary carcinoma and hyperparathyroidism
it is difficult to associate this with the amyloid
seen in islet cell tumours. Islet cells are derived
from the foregut and have much in common with
the other endocrine derivatives of this region.
It is known that islet cell tumours may produce
several peptide hormones but calcitonin has not
been identified. However, glucagon, also a low
molecular weight peptide hormone, has been
shown to have a hypocalcaemic action in the rat
(Williams, Bowser, and Henderson, 1969).

The association of the intrafollicular amyloid
deposits with the pluriglandular adenomatous
syndrome in seven of the cases may merely reflect
the long duration of disturbed calcium metaboli-
tism. It is well known that hypercalcaemia is often
asymptomatic in these families.

Thus it would appear that there are two possible
explanations for the presence of intrafollicular
amyloid in parathyroids in primary hyperparathy-
roidism: (1) a non-specific relationship with
cells capable of peptide synthesis, or (2) a more
specific association with a peptide related to
calcitonin. The latter seems more likely at present
but will remain speculative until more informa-
tion is available concerning calcitonin levels in
cases of primary and secondary hyperparathyroid-
ism, and the site of calcitonin production in the
parathyroids is identified by histochemical,
immunofluorescent, and electron microscopy
methods.

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References


