The Pathologic Assessment of Primary Hyperparathyroidism and its Impact on Therapy

A Prospective Evaluation of 50 Cases with Oil-Red-O Stain

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Normal and abnormal parathyroid glands removed surgically from 50 consecutive patients with primary hyperparathyroidism were studied by means of hematoxylin and eosin and oil-red-O stains. This was done to establish the incidence of the different pathological entities in our patient material, and to evaluate the role of the oil-red-O stain in the diagnostic histopathological process. The oil-red-O stain distinctly separates abnormally hyperfunctioning from normal or suppressed parathyroid tissue. Therefore, it is a valuable tool in the pathologic evaluation of patients with hyperparathyroidism. Chief cell adenomas were the most frequent lesions (88%), while diffuse hyperplasia was seen less commonly (10%) and carcinoma infrequently (2%). These findings justify a surgically conservative approach as the most desirable in managing patients with primary hyperparathyroidism.

THERE HAS BEEN INCREASING confusion concerning the relative prevalence of the various pathological disorders resulting in primary hyperparathyroidism. While most authors[3,4,6,11–13] agree that the predominant lesion is a solitary chief cell adenoma, some[8,10,19] claim that diffuse chief cell hyperplasia is far more common than previously suspected, justifying subtotal parathyroidectomy as the operation of choice.[16] A strong argument,[6,17] however, can be made for a "conservative" operative approach, namely excision of the adenoma and biopsy of a "normal" parathyroid, and reserving subtotal parathyroidectomy for biopsy proven hyperplasia.

Distinction between the different pathological entities rest ultimately on microscopic diagnosis. This is largely determined by evaluating parenchymal (intraparenchymal)[18] as well as stromal (interparenchymal) fat content. This distinction has been greatly facilitated by use of specific fat stains, *i.e.*, oil-red-O, whereas stromal fat is adequately evaluated in hematoxylin and eosin stained sections. Generally,[2] "normal" parathyroid tissue in the adult has ample parenchymal and stromal fat while hyperfunctioning tissue, as manifested by clinical hyperparathyroidism, is depleted of both. Although Roth and Gallagher[18] are largely responsible for demonstrating the advantages of the use of a fat stain in the pathological evaluation of cases of primary hyperparathyroidism, specific instances where this might be of clinical use have not been elucidated. This study has been designed to provide such information.

Materials and Methods

Over a three year period (1976–1979), all patients treated surgically for primary hyperparathyroidism at the Presbyterian-University Hospital were included in the study. The specimens were weighed and measured. When parathyroid tissue was identified histologically, an extra section was prepared on the cryostat, fixed in 10% formaldehyde solution, and afterwards stained with oil-red-O and counterstained with Harris' hematoxylin solution.[15] All of the remaining tissue from each case was fixed in buffered 10% formalin solution and processed in such a manner that hematoxylin and eosin (H&E) sections could be prepared from paraffin blocks. In cases judged to be adenomas, only those with either a capsular parathyroid gland or with a biopsied "normal" gland were included in the study (see below).

Employing oil-red-O stained sections, parenchymal (intraparenchymal) fat was quantitated microscopically

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TABLE 1. *Relative Incidence of Pathologic Diagnoses in 50 Patients with Primary Hyperparathyroidism*

Adenomas (total)			44 (88%)
single	43	(97.7%)	
double	1	(2.2%)	
mediastinal	1	(2.2%)	
lipoadenoma	1	(2.2%)	
Chief cell hyperplasia			5 (10%)
Carcinoma			1 (2%)
Total			50 (100%)

on a scale from 0 to 4+, 0+ representing the least, and 4+, the greatest amount. In H&E sections of the "normal" parathyroid gland, the stromal (interparenchymal) fat content was determined with the aid of a net micrometer in the eyepiece of the microscope similar to that done in a previous study.[5] The per cent stromal fat could then be calculated as a percentage of the parenchymal or interstitial tissue. In addition, whenever the stromal fat was less than 10%, step sections were obtained, and the amount of stromal fat re-evaluated. Also, in the hematoxylin and eosin sections of the adenoma cases, note was made of capsular parathyroid tissue, if present.

Fifty patients undergoing 51 operations were seen during this time period. The average age was 50.4 years with a range from 18–82. Thirty-seven patients were female (74%) while 13 (26%) were male, for a female to male ratio of 2.9:1.

In all patients, postoperative serum calciums were obtained immediately and after a significant interval (>6 months): All without exception became eucalcemic.

A total of seven surgeons performed the surgery in this series of patients. Over two-thirds (42) of the operations, however, were done by one of the authors (CGW).

Results

Over two-thirds of the cases (88%) as seen in Table 1 were classified as chief cell adenomas. Most of the patients had a single adenoma, with one being double and one mediastinal in location (see Case #1 below). Three of the adenomas were cystic, grossly. In over half of the adenomas (28), the fat stain showed little or no intracellular fat (0 plus), one-third (13) had only a slight amount (1+), while it was moderate (2+) in four (9%). Capsular parathyroid tissue was identified histologically in H&E sections in 30 of the 44 adenoma cases (68%). The oil-red-O stained sections showed abundant parenchymal fat (4+) in the capsular parathyroid gland of nine adenomas, thereby confirming the impression gained from H&E sections. This was especially helpful in five cases, since it was the only normal parathyroid tissue available for histological examination.

The mean stromal fat of 41 "normal" glands in 39 patients with adenomas was 26.9%. In 10 of these patients (26%) (average age 56.9 years), however, the stromal fat of the "normal" parathyroid gland was less than 10%, which remained constant in step sections, while the parenchymal fat content was uniformly 4+. No nodularity was seen. In those cases with capsular parathyroid tissue the stromal fat content was generally similar to that found in the "normal" tissue from the same patient.

The relative incidence of chief cell hyperplasia is given in Table 1. No case of clear cell hyperplasia was identified. Parenchymal as well as stromal fat was absent in most of the hyperplastic glands. The exceptions were as follows: a) In one case the stromal fat focally was 30–40% (similar to reports in literature[3]) with groups of chief cells of normal appearance interspersed. These cells contained ample parenchymal fat (4+), whereas in adjacent hyperplastic areas it was reduced; and b), in another hyperplastic case (Case 4), the smallest gland, although minimally enlarged, had a focal nodule, which had markedly reduced parenchymal fat while surrounding chief cells had ample fat. Three of the five cases with hyperplasia had clinical evidence of either a familial or multiple endocrine adenopathy.

In the one case of carcinoma, the gland was adherent to surrounding structures at the time of surgery and histologically had all of the findings usually associated with malignancy of the parathyroid gland including mitoses, fibrous septae, and occasional trabecular

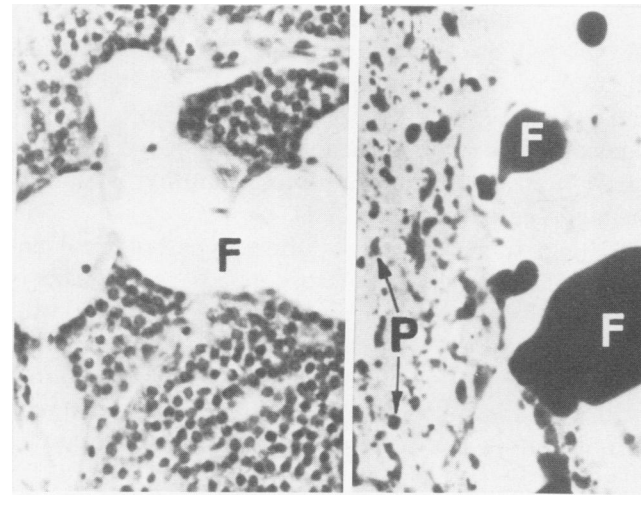

FIG. 1. (left) Photomicrograph of left lower parathyroid from Case 1. Note relationship of stromal fat (F) to chief cells (H&E, ×265). (right) Same gland stained with oil-red-O: 4+ parenchymal fat (P). Stromal fat globule (F) also demonstrated (×265).

arrangement of the neoplastic chief cells. This patient is asymptomatic and eucalcemic one year after wide excisional surgery.

The following case reports, although representative of the patients in this study, are particularly selected to illustrate the usefulness of the fat stain in resolving problems in histologic evaluation and clinical management.

Case Reports

Recognition of Normal Parathyroid Gland

Case 1: (R.T.). This 25-year-old man was found to be hypercalcemic (calcium 12.5 mg/dl) on a routine biochemical screen. A serum parathormone (PTH) level was inappropriately elevated. Neck exploration yielded four parathyroid glands, three normal in appearance and the fourth slightly enlarged (5 mm), compared to the others. The enlarged gland was removed and another biopsied. The stromal fat of both glands was ample (40–50%), while their parenchymal fat content was high (4+), indicating both to be normal or suppressed glands (Fig. 1). Postoperatively, the serum calcium remained elevated. Subsequent selective venous catheterization demonstrated a step-up in the PTH concentration in the right thymic vein, and mid mediastinal lesion was demonstrated by selective arteriography. Using a mediastinotomy approach, a 21.7 g adenoma was located within the arch of the aorta and removed. The tumor's parenchymal fat was minimal (0+).

Comment. With a fair degree of certainty, we were able to predict that the two parathyroid glands removed at the time of the first operation, even though one was marginally enlarged, were not abnormal largely on the basis of the results of the fat stain. This was confirmed by persistent hypercalcemia postoperatively. In contrast the intracellular fat was abnormal (*i.e.*, greatly diminished) in the adenoma, removed subsequently. The patient has remained eucalcemic (24 months to date).

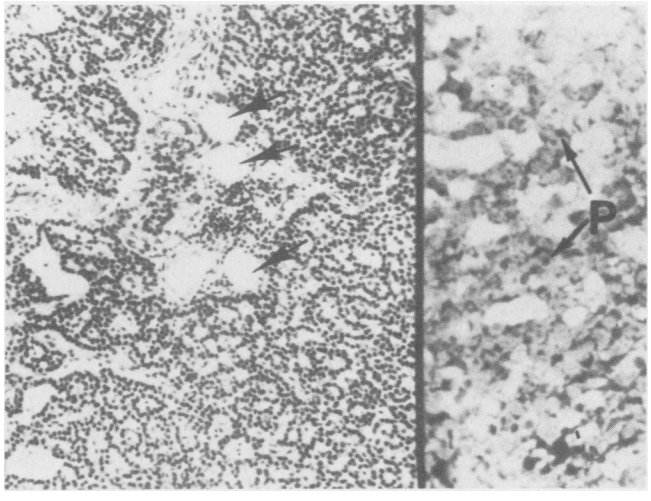

FIG. 2. (left) Photomicrograph of right superior gland from Case #2. Note stromal fat (5%) (arrows). Spaces without arrows are glands (H&E; ×105). (right) Same gland stained with oil-red-O: 4+ parenchymal fat (P) (×265).

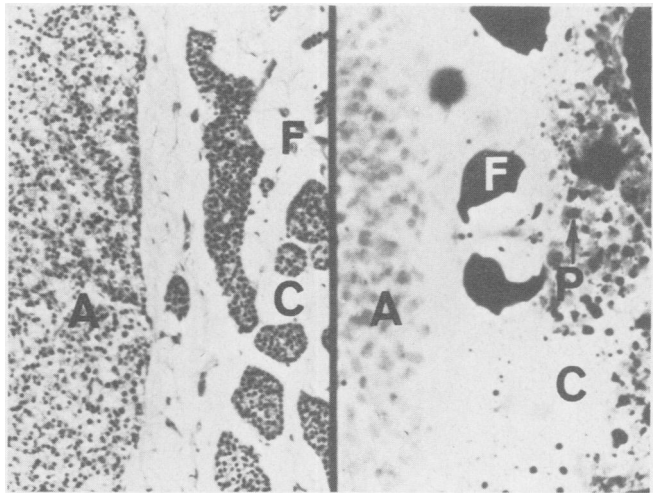

FIG. 3. (left) Photomicrograph of adenoma (A) from Case 3 with capsular tissue (C) with abundant stromal fat (F) (H&E; ×105). (right) Same section stained with oil-red-O. Parenchymal fat (P) in capsular parathyroid (C), 4+; 0+ in adenoma (A). Stromal fat: (F) (×265).

"Pseudohyperplasia" of a Normal Gland

Case 2: (D.K.). For over a year, this 34-year-old woman had asymptomatic serum calcium elevations up to 11.6 mg/dl. The serum PTH level was inappropriately elevated. The patient's neck was explored and a 700 mg adenoma was removed from the left side. A 0.3 cm "normal" right superior parathyroid was also removed, whose stromal (interparenchymal) fat content was uniformly 5% in serial step sections while the parenchymal fat was 4+. The parenchymal fat content of the adenoma was 1+ (Fig. 2). The patient has remained eucalcemic postoperatively.

Comment. Even though the right superior parathyroid was small grossly, microscopically, in serial step H&E sections, it appeared abnormal, due to the presence of only 5% stromal fat. The parenchymal fat stain conclusively differentiated between the two specimens, however, thereby indicating that the tumor was actively functioning while the contralateral parathyroid gland most likely was not. The fat stain was of crucial diagnostic help in this case and nine others like it in confirming that the impression of hyperplasia of the "normal" gland was spurious. Postoperatively the calcium returned to normal levels, and has remained so for 24 months.

Capsular Parathyroid Tissue

Case 3: (L.C.). This 68-year-old woman had a three year history of hypercalcemia, discovered incidentally. The serum PTH level was inappropriately elevated. The patient's neck was explored and a 1.3 g tumor of the left inferior gland was found. Intraoperative parenchymal fat as well as H&E stains demonstrated normal capsular parathyroid tissue. The latter had ample intracytoplasmic

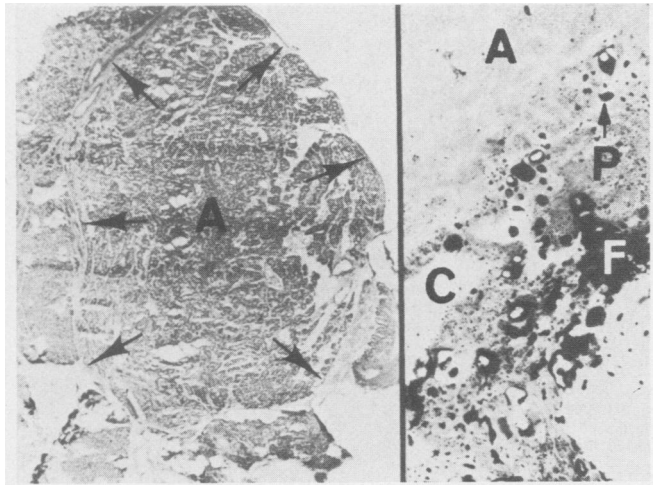

FIG. 4. (left) Photomicrograph of frozen section preparation of left superior gland from Case 4. Note small microadenoma (A), outlined by a fibrous capsule (arrows), without stromal fat (H&E: ×43). (right) Oil-red-O stain: Parenchymal fat (P) in adenoma (A), 0+; surrounding gland (C), 4+. Stromal fat: (F) (×126).

fat while the adenoma had none (Fig. 3). The histologic diagnosis was a chief cell parathyroid adenoma of the left inferior gland. The postoperative serum calcium levels have remained within normal limits (12 months).

Comment. Without the vivid demonstration of normal capsular parathyroid tissue in the H&E stained sections, the diagnosis would have been in doubt from a histologic point of view since no other parathyroid tissue was identified. The oil-red-O sections were largely confirmatory but especially helpful at the time of operation. The histologic diagnosis of adenoma seems to be confirmed by the persistent eucalcemia, postoperatively.

Recognition of Hyperplastic and Nonhyperplastic Areas in Hyperplasia

Case 4: (B.C.). This 47-year-old woman had progressive asymptomatic hypercalcemia for a six year period, with the serum calcium ranging up to 11.3 mg/dl and an inappropriately elevated serum PTH. Intraoperatively, four parathyroid glands were enlarged, the right more than the left. Three and one-half were removed, with a combined weight of 245 mg. Histologically, glands from the left side of the neck were essentially normal with 30% stromal fat and 4+ parenchymal fat, except for a small nodule of chief cells in the upper gland which contained no parenchymal fat (Fig. 4). The other gland on this side even in serial sections was normal. Histologically, the two glands on the right side contained large nodular aggregates of chief cells of varied appearance which uniformly contained no intracellular fat. Postoperatively the patient became eucalcemic and has remained so.

Comment. Unequal involvement of parathyroid glands in chief cell hyperplasia is demonstrated. The fat stain was crucial in defining hyperfunctioning nodules in an otherwise normal gland, which was especially helpful in the frozen section preparation during surgery, at which time the diagnosis of hyperplasia could not have been made on the basis of H&E sections alone, which confirms previous reports[3] that occasional hyperplastic glands may be only minimally involved in the adenomatous hyperplastic process.

Discussion

The relative frequency of adenomas and hyperplastic parathyroid glands encountered in this three year prospective study of patients treated surgically for primary hyperparathyroidism approximates that reported by Castleman, et al., in a larger series extending over more than five decades.[3] This is significantly at variance with the preponderance of hyperplastic lesions recently reported.[8,10,16,19] The reasons for this apparent high incidence of hyperplasia may be several:[3] inclusion of an unusually large number of familial cases who usually have diffuse hyperplasia; the inclusion of asymptomatic patients who may have subtle changes in the parathyroid glands making it difficult to distinguish between adenoma, mild chief cell hyperplasia, or normal glands; lastly, a possible source of error, well known but infrequently mentioned[1,2,14] is the difficulty in consistently establishing an accurate pathologic diagnosis on the basis of H&E sections alone. Several of our case reports demonstrate this difficulty and the advantages afforded by the parenchymal fat stain in the differential diagnosis of these entities. The diagnosis usually is not conclusive with any one technique but depends on a composite review of intraoperative gross morphology, routine H&E and oil-red-O microscopic findings, and in the final analysis on the evaluation of postoperative serum calciums.

One potential source of error in histological diagnosis, also not widely recognized, is the uniform decrease of stromal fat to less than 10% in occasional "normal" glands associated with single adenomas in adult patients contrasted to an anticipated "normal" stromal fat content of 40–50%.[2] This was observed in one-quarter (26%) of our cases with solitary adenoma. Even though this might conceivably be construed as evidence for hyperplasia, as recently reported,[12] the fact that all had ample parenchymal fat (4+) indicates the "normal" nature of these glands, a fact eventually corroborated by persistent postoperative eucalcemia in all our patients with single adenomas. The finding of decreased stromal fat or "pseudohyperplasia" has also recently been observed in a series of autopsied adult patients,[5] without clinical or pathologic evidence of hyperfunctioning parathyroid tissue. These findings, therefore, in both surgical and autopsied cases indicate that variation in stromal fat must be viewed with

caution when making functional predictions on the basis of histology alone. Only when the gland is grossly enlarged, even if slight, and the parenchymal fat in the chief cells decreased, may decreased stromal fat be used as additional evidence for hyperfunction. The marked histological uniformity in step sections, in at least these "pseudohyperplastic glands," is at variance with the report of Grimelius, et al.,[9] who found marked variation in serially sectioned parathyroid glands of patients with adenomas.

Generally,[17] the parathyroid glands associated with single adenomas are described as "suppressed" or "atrophic." Recent work[7,12] does not support this contention based largely on histological appearances. Even though our histologic observations support the non-atrophic state of the "normal" gland, we would urge caution in the histological interpretation of H&E sections, as stated above. Reliance on the results of the fat stain seems more reliable.

Nevertheless, in the majority of cases the fat stain is merely confirmatory of the H&E sections. In a small, but important group, however, this stain provides crucial and unequivocal information, not available from examining H&E sections. It aids in the differentiation between normal and equivocally normal glands as seen in Cases 1 and 2. Its greatest use, however, is in case of hyperplasia, as illustrated in Case 4, where unequal involvement makes histological interpretation most difficult. With this technique focally hyperfunctioning chief cells, either in diffuse or nodular form, can readily be appreciated. Since the completion of this study, similar variable fat distribution was seen in another case of chief cell hyperplasia.

Also the fat stain is most useful in those adenoma cases (five in total), where for various reasons a normal gland was not identified (Case 3), but the diagnosis could still be made with an intraoperative fat stain by demonstrating ample parenchymal fat in capsular parathyroid tissue. Capsular parathyroid can frequently (68%) be identified in adenomas stained with H&E. For technical reasons, however, the H&E stained frozen section preparation is less accurate in demonstrating capsular parathyroid, a much greater accuracy being achieved with oil-red-O stain.

The overall importance of accurately categorizing the pathology of diseased parathyroid glands is its impact on therapy. Because of the preponderance of solitary adenomas in our series and most other series, we generally follow the approach recommended by Roth, et al.[17] The excision of one abnormal gland and documentation by biopsy of a coexistent "normal" gland is by and large curative, as adenomas are usually solitary. Other authors, due to the finding of a high incidence of "hyperplasia" in their series, recommend biopsy of all parathyroid glands[14] or subtotal parathyroidectomy in every case.[16] Based on our experience and those of others[6,17] we consider these two approaches to be needlessly excessive, time consuming, and without apparent benefit to the patient.

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