A Density Test for the Intraoperative Differentiation of Parathyroid Hyperplasia from Neoplasia

CHIU-AN WANG, M.D.,* SIDNEY V. RIEDER, PH.D.†

From the Departments of Surgery and Clinical Chemistry of the Massachusetts General Hospital, and Harvard Medical School, Boston, Massachusetts

A simple, instant, and reliable test called the Density Test, based on the difference in density between the normal and the diseased parathyroid tissue, was performed intraoperatively in 73 patients with primary hyperparathyroidism. Whereas the normal parathyroid tissue floated, the diseased tissue invariably sank in a mannitol solution with a density range between 1.049 and 1.069. With the aid of this test, a diagnosis of adenoma was made in 66 patients and of primary hyperplasia in the remaining seven. In every case the diagnosis was subsequently confirmed. Forty-two of the 66 patients with an adenoma (64%) had a unilateral exploration of the neck. The Density Test saved these patients from an unnecessary contralateral exploration by the finding of an adenoma and a normal second gland in the same side of the neck. These data show that the Density Test is useful in the intraoperative diagnosis of a diseased from a normal parathyroid tissue. Tissue that sinks within the density range of 1.049 and 1.069 is without exception diseased and should therefore be either partially or completely excised even if the gland is of average size or only of slight enlargement. If it does not sink, it is virtually certain to be normal and should be spared. The Density Test provides a valuable clue in the differentiation of primary parathyroid hyperplasia from neoplasia.

EVER SINCE THE FIRST recognition of primary hyperplasia as a pathologic entity of hyperparathyroidism,[1,4] its differentiation from a normal or an adenomatous parathyroid gland at the time of surgical exploration has posed a difficult problem to both surgeon and pathologist. The diagnosis of hyperplasia or adenoma can seldom be made on the basis of a single enlarged gland, and another gland is invariably needed for comparison. If this second gland is normal, the third, fourth, and sometimes, even a fifth gland are likely to be free of the disease. In this case, the enlarged gland is virtually certain to be a neoplasm, either the common adenoma or one of the rare carcinomas.[3,9] The so-called "double adenomas," if they indeed exist, are extremely rare, and we have not encountered a single case since 1958.‡ In contrast, if the second gland is also enlarged, involvement of all the other glands is certain, and primary hyperplasia may be expected. If, however, the second gland is averaged in size or slightly enlarged, difficulty arises as to whether it is diseased or not.

We have recently developed an objective test called the Density Test for the intraoperative differentiation of a normal and a diseased gland. On the basis of the results of this test, we were invariably able to differentiate a diseased from a normal gland and, hence, to make the diagnosis of either neoplasia or hyperplasia at surgery.

Materials and Methods

A sliver of tissue between 2 and 3 mm in thickness was taken from the central portion of an enlarged gland and another sliver, with the periglandular fat completely removed, from a gland of average size or of only slight enlargement. The slivers were then placed in a test tube with about 2 ml of 25 per cent mannitol solution (Fig. 1).§ By gradually diluting the mannitol solution with approximately 0.5 ml of water, a density was soon reached at which one or both tissues began to sink to the bottom. If only one tissue sank in this solution, there was clearly a difference in density, indicating that only one gland was involved with the disease and that diagnosis was virtually cer-

* Associate Clinical Professor of Surgery, Harvard Medical School, Visiting Surgeon, Massachusetts General Hospital, Boston, Massachusetts.

† Chief, Clinical Chemistry Laboratories, Massachusetts General Hospital, Boston, Massachusetts.

Reprint requests: Chiu-an Wang, M.D., Massachusetts General Hospital, Boston, Massachusetts 02114.

Submitted for publication: March 28, 1977.

‡ Prior to 1958, glands from 13 of 217 patients with primary hyperparathyroidism were diagnosed as double adenomas. Of these, six proved subsequently to be primary hyperplasia and seven have not been conclusively substantiated.

§ Mannitol injection USP 25% (12.5 g in 50 ml), Manufacturer: "Invenex," Chagrin Falls, Ohio 44022.

0003-4932-78-0100-0063-0075

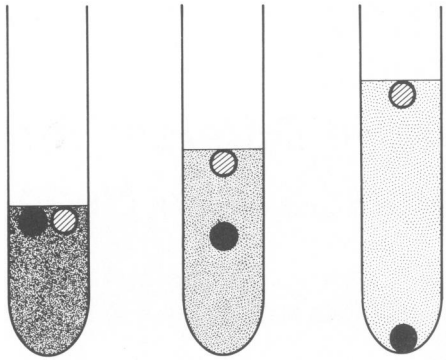

FIG. 1. Schematic Presentation of Density Test (See text). Cross-hatched circle = floats solid circle = sinks.

tain to be neoplasia, usually an adenoma. If both tissues sank, tissue from the remaining glands would likewise sink, the disease process was generalized, and the diagnosis was primary hyperplasia.

The density of the mannitol solution was determined by weighing 1 ml of the solution delivered into a tared weighing bottle from a calibrated 1 ml pipet. All measurements were made using a semimicro-analytic balance at room temperature of 23°. A slight change in temperature had no noticeable effect. The overall error in measurement was about ±0.002 gm/ml at the maximum. After the Density Test, the same tissues were studied by frozen section with fat stain and permanent histologic examination.

Clinical Data

The Density Test was performed intraoperatively in 73 patients with proven primary hyperparathyroidism. Nineteen of the patients were men and 54, women (Fig. 2). Sixty-one (82%) were between the ages of 40 and 69; the youngest patient was 14 and the oldest, 75.

In 52 patients, 2 ml of 25% mannitol solution diluted with about 0.5 ml of water as described above was

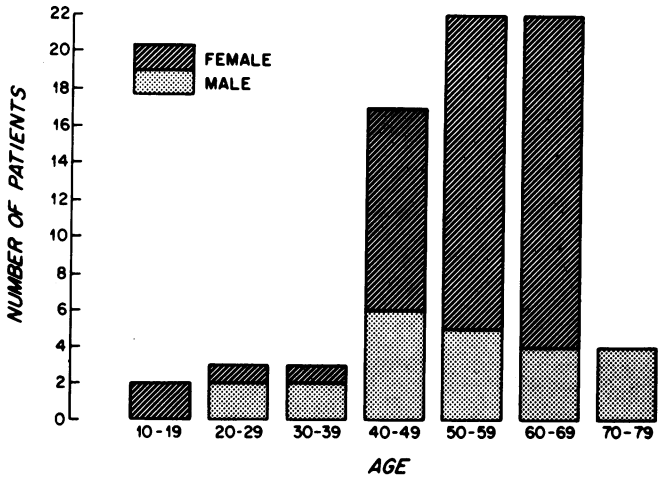

FIG. 2. Sex and Age Distribution of 72 Patients Who Underwent Density Test.

used for the test (Table 1). In 46, the tissue from the enlarged gland sank readily in the solution and that from the gland of normal size floated; all 46 patients proved to have an adenoma. In the remaining six patients, the tissue from two or more glands sank almost simultaneously in the above solution, and all six patients were found to have primary hyperplasia.

The density of the solution was determined in 28 cases (Fig. 3). It was found to be within a range of 1.049 to 1.069; the mean at which tissue from the enlarged gland sank was 1.055 ± 0.006 SD. In 12 others, tissue from the gland of normal size had remained afloat until the solution was further diluted with approximately 8 ml of water. Under these conditions, a new density was reached ranging from 1.010 to 1.038, with a mean of 1.017 ± 0.008 SD. These findings clearly indicate that there is a marked difference in density between the diseased and the normal parathyroid tissue.

For the remaining 21 patients, the density of the solution had been calibrated at 1.069 to 1.050 prior to the test (Table 2). This density was chosen as the lowest possible level at which a diseased parathyroid tissue would sink even when the degree of hyperparathyroidism was relatively mild. Twenty patients in this group had an adenoma and one had primary hyperplasia.

In the entire group of 73 patients, 66 had adenomas and seven, primary hyperplasia. Unilateral exploration was performed in 42 in whom an adenoma and a normal second gland were uncovered in the same side of the neck. Bilateral exploration was performed in 31 patients. Seven of these had primary hyperplasia; of the remaining 24, either the diseased gland was located in the side opposite that of the initial exploration or surgery was performed to verify the accuracy of the Density Test.

The diagnosis in each case was subsequently confirmed by the postoperative serum calcium determination, and in 44 patients, the plasma parathormone, preoperatively found to be elevated, returned to a normal range immediately after surgery.

Discussion

It is well known that stromal fat cells begin to appear in a normal parathyroid gland after puberty and continue to increase numerically until a person reaches 40. Thereafter, they diminish somewhat in number but never completely disappear even in later years.[2] Intracellular fat granules are generally abundant in a normal gland and are even more numerous in a normal gland suppressed by an adenoma.[8] In sharp contrast, there are few or no stromal fat cells or intracellular fat granules in a diseased parathyroid gland.* The Density

* With a mild degree of hyperparathyroidism, a few fat cells or fat granules may be present in a diseased gland. Their presence accounts for the relatively low density of some of these diseased tissues.

TABLE 1. *Clinical Data of Density Test Performed in 52 Patients with Primary Hyperparathyroidism*

Case No.	Gland(s)		Density of Solution	Diagnosis	Exploration of the Neck	Serum Calcium†		Plasma Parathormone‡	
	Floated*	Sank*				Preoperative	Postoperative	Preoperative	Postoperative
1	RU,LU LL	RL	1.050	Adenoma (RL)	Bilateral	11.8	8.6	12	2.5
2	RU	RL	1.055	Adenoma (RL)	Unilateral	11.6	8.6	38	3.5
3	LU RL,LL	RU	—	Adenoma (RU)	Bilateral	11.6	8.4	46	3.5
4	None	RL,LU	1.051	Hyperplasia	Bilateral	11.4	8.0	33	3.5
5	RU,LU LL	RL	—	Adenoma (RL)	Bilateral	10.6	8.4	20	5.0
6	LU LL	RU	—	Adenoma (RU)	Bilateral	12.8	8.0	41	4.5
7	LL	LU	1.055	Adenoma (LU)	Unilateral	12.2	7.8	27	5.0
8	RU	RL	—	Adenoma (RL)	Unilateral	11.7	9.2	23	2.5
9	RU	RL	1.051	Adenoma (RL)	Unilateral	11.7	8.8	20	5.0
10	RU	RL	—	Adenoma (RL)	Unilateral	11.0	8.8	26	5.0
11	None	LU,RU LL,RL	1.054	Hyperplasia	Bilateral	10.9	7.3	15	2.5
12	RL	RU	—	Adenoma (RU)	Unilateral	12.4	7.8	40	7.5
13	RU	RL	—	Adenoma (RL)	Unilateral	12.0	8.5	11	5.0
14	RL,LL	RU	—	Adenoma (RU)	Bilateral	12.0	8.4	21	5.0
15	RL	RU	—	Adenoma (RU)	Unilateral	13.0	9.3	45	3.0
16	None	RU,RL LU,LL	1.060	Hyperplasia	Bilateral	13.8	9.9	42	3.5
17	RL	RU	—	Adenoma (RU)	Unilateral	15.0	8.8	—	—
18	RL	RU	—	Adenoma (RU)	Unilateral	12.0	8.9	12	8.5
19	RL	LU	1.050	Adenoma (LU)	Bilateral	10.7	8.5	15	3.5
20	RL	LU	—	Adenoma (LU)	Bilateral	11.0	7.9	26	5.0
21	RL	RU	1.058	Adenoma (RU)	Unilateral	12.5	9.3	—	—
22	RL	RU	—	Adenoma (RU)	Unilateral	12.7	6.9	60	9.0
23	RL	RU	—	Adenoma (RU)	Unilateral	11.0	8.9	14	5.0
24	None	RU,LU RL,LL	1.058	Hyperplasia	Bilateral	12.0	8.0	11	4.0
25	RL	RU	—	Adenoma (RU)	Unilateral	11.4	9.3	13	5.0
26	RU RL,LL	LU	—	Adenoma (LU)	Bilateral	11.3	8.8	20	2.5
27	RU,RL LL	LU	—	Adenoma (LU)	Bilateral	12.0	8.0	—	—
28	RL	RU	—	Adenoma (RU)	Unilateral	12.6	8.8	20	3.0
29	None	RU,RL LU,LL	1.057	Hyperplasia	Bilateral	11.5	9.2	14	1.0
30	LL RU	LU	—	Adenoma (LU)	Bilateral	12.0	8.6	—	—
31	RL	LL	—	Adenoma (LL)	Bilateral	12.5	8.8	—	—
32	LU	LL	—	Adenoma (LL)	Unilateral	12.2	7.9	13.5	10
33	RU,LU	RL	—	Adenoma (RL)	Bilateral	11.6	8.7	—	—
34	RL	RU	1.064	Adenoma (RU)	Unilateral	11.0	9.0	14	5.0
35	None	RU,RL LU,LL	1.056	Hyperplasia	Bilateral	12.0	8.4	—	—
36	RU	RL	1.064	Adenoma (RL)	Unilateral	12.6	8.0	—	—
37	RU	RL	1.064	Adenoma (RL)	Unilateral	11.0	8.0	—	—
38	RU,RL LU	LL	1.062	Adenoma (LL)	Bilateral	12.0	8.0	—	—
39	RU,RL	LL	1.048	Adenoma (LL)	Bilateral	12.0	8.0	—	—
40	RU	RL	1.063	Adenoma (RL)	Unilateral	12.2	8.6	—	—
41	LL	RL	1.055	Adenoma (RL)	Bilateral	12.0	8.6	—	—
42	RL	RU	1.060	Adenoma (RU)	Unilateral	11.6	8.8	14	5.0
43	LU	LL	1.059	Adenoma (LL)	Unilateral	13.0	9.6	15	5.0
44	LU	RU	1.059	Adenoma (RU)	Bilateral	11.0	8.8	13	5.0
45	LU	LL	1.057	Adenoma (LL)	Unilateral	12.0	8.6	—	—
46	LL	LU	1.055	Adenoma (LU)	Unilateral	12.6	8.0	—	—
47	RU	RL	—	Adenoma (RL)	Unilateral	11.0	8.3	—	—
48	LL	LU	1.055	Adenoma (LU)	Unilateral	11.0	8.2	—	—
49	RU	LL	1.053	Adenoma (LL)	Bilateral	11.7	9.0	—	—
50	RL	RU	1.062	Adenoma (RU)	Unilateral	11.9	9.1	—	—
51	LU	LL	1.061	Adenoma (LL)	Unilateral	11.8	9.9	—	—
52	RU	RL	1.057	Adenoma (RL)	Unilateral	11.9	8.8	19	5.0

* In mannitol solution. (See text)
† Normal value = 8.4 to 10.4 mg/100 ml.
‡ Normal value = <10 microliter equivalent.

RU = Right upper gland.
RL = Right lower gland.
LU = Left upper gland.
LL = Left lower gland.

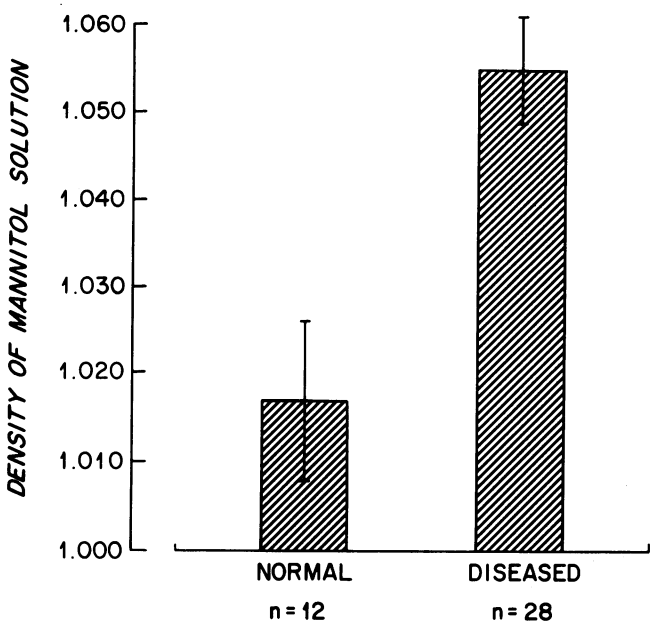

FIG. 3. Density Range in Which Parathyroid Tissue Sinks.

Test herein described is based on the marked difference between the fat content of the normal and that of the diseased parathyroid. With little or no fat, the diseased parathyroid tissue is heavier per unit volume than the normal tissue and would invariably sink in a solution with a density less than its own but still greater than that of the normal tissue. It is clear from this study that the Density Test can aid the surgeon in determining whether or not a parathyroid gland is diseased at the time of surgery.

It goes without saying that a surgeon must recognize the parathyroid gland when he sees one, although occasionally, he may confuse a fat lobule, a lymph node, or even a thyroid nodule with a parathyroid gland. However, a fat lobule may be recognized by its buoyancy in saline solution as opposed to parathyroid tissue which is heavier and will sink, and the firm-to-hard consistency of the lymph node and the thyroid distinguishes them from the parathyroid which is soft and malleable. Examination by frozen section can generally resolve uncertainty about the diagnosis.

In recent years, there has been disagreement over the surgical management of patients with primary hyperparathyroidism.[10] Some surgeons favor routine bilateral exploration of the neck[5,6] and excision of three and a half glands in every case.[7] Others advocate that surgery should always be performed on the basis of the pathologic entity of the disease.[3,9] Such disagreement stems from the difficulty in determining whether a gland

TABLE 2. *Clinical Data of Density Test Performed in 21 Patients with Primary Hyperparathyroidism*

Case No.	Gland(s) Floated*	Gland(s) Sank*	Density of Solution	Diagnosis	Exploration of the Neck	Serum Calcium† Preoperative	Serum Calcium† Postoperative	Plasma Parathormone‡ Preoperative	Plasma Parathormone‡ Postoperative
1	RL	LL	1.050	Adenoma (LL)	Bilateral	10.8	8.0	10	2.5
2	RL	RU	1.050	Adenoma (RU)	Unilateral	10.7	8.1	10.5	3
3	LL	LU	1.050	Adenoma (LU)	Unilateral	11.3	8.7	11	3
4	RL	RU	1.050	Adenoma (RU)	Unilateral	11.3	8.7	39	6
5	RL	RU	1.050	Adenoma (RU)	Unilateral	11.4	8.6	22	2.5
6	RU	RL	1.050	Adenoma (RL)	Unilateral	11.5	8.8	9.5	5.5
7	RL	RU	1.050	Adenoma (RU)	Unilateral	10.8	8.7	—	—
8	LL	RL	1.050	Adenoma (RL)	Bilateral	11.2	8.5	10	3
9	RL	RU	1.050	Adenoma (RU)	Unilateral	11.1	8.7	12.5	5
10	LU LL	RL	1.050	Adenoma (RL)	Bilateral	11.0	8.5	17	5
11	RU	RL	1.050	Adenoma (RL)	Unilateral	12.3	9.2	15	5.5
12	RL	RU	1.050	Adenoma (RU)	Unilateral	12.0	9.2	—	—
13	None	LU,LL RL	1.050	Hyperplasia	Bilateral	12.0	10.0	—	—
14	LL	LU	1.050	Adenoma (LU)	Unilateral	13.0	8.6	—	—
15	RU,LL LU	RL	1.049	Adenoma (RL)	Bilateral	10.4	8.6	—	—
16	LL	LU	1.049	Adenoma (LU)	Unilateral	12.3	8.9	—	—
17	RU RL	LU	1.049	Adenoma (LU)	Bilateral	12.0	8.8	—	—
18	RL	RU	1.049	Adenoma (RU)	Unilateral	10.8	8.6	10.5	3.5
19	RL	LU	1.049	Adenoma (LU)	Bilateral	11.1	8.2	—	—
20	RL	RU	1.049	Adenoma (RU)	Unilateral	12.0	9.0	44	5.5
21	RU RL	LU	1.049	Adenoma (LU)	Bilateral	11.0	9.5	—	—

* In mannitol solution. (See text)
† Normal value = 8.4 to 10.4 mg/100 ml.
‡ Normal value = <10 microliter equivalent.
RU = Right upper gland.
RL = Right lower gland.
LU = Left upper gland.
LL = Left lower gland.

of normal size or slight enlargement is diseased or not. We believe that this problem can generally be resolved with the aid of the Density Test and that there is seldom a need for routine bilateral exploration or radical resection of the glands unless the diagnosis clearly is primary hyperplasia. Forty—two (64%) of the 66 patients with the diagnosis of adenoma in this study were spared an unnecessary exploration of the opposite side of the neck because of an unequivocal diagnosis of an adenoma.

Critics may ask why another test is needed. It is true that a pathologic diagnosis can usually be obtained by frozen section examination and that an experienced pathologist can generally determine normality or disease in a gland by estimating the aggregate number of stromal fat cells and fat granules present in the tissue. We have found, however, that because fat cells and granules are not uniformly distributed, they may be scanty in one area and abundant in another. The diagnosis can be tenuous.* In the hands of a less experienced pathologist, diagnostic error may be expected even more frequently. With the Density Test, determination of the total fat content of the tissue eliminates the potential for error. Still another advantage of the Density Test is the ease with which it can be executed. It can confirm the diagnosis within two minutes as opposed to the 15 or 20 minutes required for an examination by frozen section.

Because the primary purpose of the Density Test is to demonstrate the difference in the density of the tissues under consideration, calibration of the exact density of the mannitol solution, although of academic interest, is not absolutely essential in each case. To have the mannitol solution readily available for the performance of the test, however, we have prepared one with a density of 1.049 to 1.050 and have found that at this level the diseased glandular tissue invariably sinks even if the degree of hyperparathyroidism involved is mild.

The accuracy of the Density Test is not affected by the size, weight, or volume of the parathyroid tissue. Nonetheless, a sliver of tissue should be no less than 2 mm in diameter. It should be free of periglandular fat, and if a rim of normal tissue is present in the diseased gland, it should be removed before the test.

Mannitol solution is found to be most satisfactory for the test because it neither alters nor damages the structure of the tissue. Thus, after the Density Test, the same sliver of tissue can be used for permanent histologic examination. Another point in its favor is that the mannitol solution is readily available in high concentration.

* In seven instances in this study, a normal gland was interpreted as abnormal by the pathologist contrary to the findings of normality by the Density Test. Each gland was subsequently confirmed to be normal.

References

1. Albright, F., Bloomberg, E., Castleman, B., and Churchill, E. D.: Hyperparathyroidism Due to Diffuse Hyperplasia of All Parathyroid Glands Rather than an Adenoma of One. Clinical Study in Three Such Cases. Arch. Intern. Med. 54:315, 1934.
2. Castleman, B. and Mallory, T. B.: The Pathology of the Parathyroid Gland in Hyperparathyroidism. Am. J. Pathol. 11:1, 1935.
3. Cope, O.: The Story of Hyperparathyroidism at the Massachusetts General Hospital. N. Engl. J. Med. 274:1174, 1966.
4. Cope, O. Keynes, W. M., Roth, S. I. and Castelman, B.: Primary Chief-cell Hyperplasia of Parathyroid Glands. A New Entity in the Surgery of Hyperparathyroidism. Ann. Surg. 148:375, 1958.
5. Esselstyn, C. B., Jr., Levin, H. S., Eversman, J. J., et al.: Reappraisal of Parathyroid Pathology in Hyperparathyroidism. Surg. Clin. North Am. 54:443, 1974.
6. Haff, R. C. and Ballinger, W. F.: Causes of Recurrent Hyperparathyroidism After Parathyroidectomy for Primary Hyperparathyroidism. Ann. Surg. 173:884, 1971.
7. Paloyan, E., Lawrence, A. M., Baker, W. H. and Straus, F. H.: Near-total Parathyroidectomy. Surg. Clin. North Am. 49:43, 1969.
8. Roth, S. I. and Gallagher, M. J.: The Rapid Identification of "Normal" Parathyroid Glands by the Presence of Intracellular Fat. Am. J. Pathol. 84:521, 1976.
9. Wang, C. A.: Surgery of the Parathyroid Glands, *In* Welch, C. E., and Hardy, J. D. (eds), Advances in Surgery, Chicago, Year Book Medical Publishers, Inc., vol. 5, 1971, pp. 109–127.
10. Wang, C. A., Potts, J. T., Jr. and Neer, R. M.: Controversy of Parathyroid Surgery. Excerpta Medica International Congress Series No. 346:82, 1974.