

Vitamin D Activity of Normal and Neoplastic Human Breast Tissue.* (28483)

GILBERT S. GORDAN[†] AND DAVID SCHACHTER[‡]

*Department of Medicine, Columbia University College of Physicians and Surgeons,
and the Presbyterian Hospital, New York City*

Hypercalcemia is a frequent, serious complication of disseminated carcinoma of the female breast. Its occasional occurrence in the absence of demonstrable osseous metastases suggests the possibility of a hypercalcemia-inducing substance in the tumor. Breast cancer tissue, parathyroid tissue and Vit. D pellets implanted against the calvarium of the rat have been shown to lyse the adjacent bone, but normal breast tissue does not(1,2). Indirect evidence suggests that the osteolytic substance in breast cancer resembles Vit. D rather than parathyroid hormone: (1) the hypercalcemia of breast cancer usually responds to cortisone while that of hyperparathyroidism rarely does; (2) renal tubular reabsorption of phosphate is normal in patients with breast cancer, but is usually low in patients with hyperparathyroidism; (3) patients with breast cancer do not show the pathognomonic roentgen changes in the phalanges found in 22% of patients with hyperparathyroidism(2).

Methods. Vitamin D activity of tissues was assayed by the method of Schachter, Kimberg and Schenker(3), based on the dependence of intestinal transport of calcium on Vit. D and sensitive to 0.2 IU (0.005 μ g). Female breast cancer, normal male and female breast and other tissues were obtained at autopsy,[§] dissected from adjacent fat, and weighed aliquots placed in tared aluminum planchets. Each aliquot was fed to a rachitic, albino, male rat of the Sherman strain which had been starved overnight. Rats were

fed the USP rachitogenic diet No. 2|| from weaning and had been on this diet 5 or more weeks before the assay. A group of 5 animals was used to assay each tissue level, Vit. D standard or control. Three days after treatment, each rat was decapitated and its duodenum removed and sliced by hand using a Stadie blade. 130-150 mg of pooled slices from each group were placed in each of 5 chilled Warburg vessels containing 2.5 ml of the following medium: fructose (0.02 M), CaCl_2 (1×10^{-4} M), NaCl (0.145 M), KCl (0.004 M), sodium phosphate buffer of pH 7.4 (0.004 M), and Ca^{47} (Abbott Laboratories, Oak Ridge, Tenn.) sufficient to give 2000-6000 cpm/ml in a well-type scintillation counter. The gas phase was O_2 in 4 vessels and N_2 in the fifth. As controls and standards, duodenal slices were similarly prepared from untreated rachitic rats and from animals dosed with 0.2, 2.0 and 20 IU Vit. D_3 by gastric tube. Tissues were incubated 1 hour at 37° in a Warburg bath shaking at 120 cycles per minute. Tissues were then removed, blotted, weighed and radioactivity counted. Vit. D-activity of the tested tissues was calculated by interpolation of oxygen-dependent counts (O_2 minus N_2) between those from animals treated with standard doses of Vit. D_3 using a semilogarithmic scale (log dose *vs* oxygen-dependent cpm).

Results and discussion (Table I). Four of 6 primary breast cancers from hypercalcemic patients contained Vit. D-activity equivalent to 3.5-8.5 IU/g of tissue. Another contained no detectable activity (<1 IU/g) and the other approximately 100 IU/g. Three breast cancers from normocalcemic women contained similar amounts 3.5-5.0 IU/g. Activity of all 9 breast cancers averaged 15 ± 10 IU/g (mean $\pm$ SE). Six of 7 specimens of normal female breast contained

* Research supported by U. S. Public Health Service grant.

[†]On sabbatical leave from Univ. of California Medical Center, San Francisco.

[‡]Recipient of Investigatorship of Health Research Council of City of New York.

[§]We are indebted to Dr. Jacob Furth and the Staff of the Dept. of Pathology, Francis Delafield Hospital, for making these tissues available to us.

|| Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE I. Vitamin D Activity of Human Tissues Obtained at Autopsy.*

No.	Group	Vit. D activity, IU/g
9	Breast cancer	15 $\pm$ 10†
6	—Hypercalcemia	21 $\pm$ 11
3	—Normocalcemia	4.5 (3.5, 5.0, 5.0)
11	Normal breast	66 $\pm$ 14
7	—Female	55 $\pm$ 20
4	—Male	68 $\pm$ 26
2	Gynecomastia	0.6 (0, 1.3)
2	Bronchogenic carcinoma	0
3	Adipose tissue	8.1 (3.1, 6.3, 15)
1	Liver	>100

* All but 2 patients had cancer of various organs. The breast tissue was histologically uninvolved in the Normal Breast group.

† Mean $\pm$ S.E.

9.3-100 IU/g; 4 of these had 64-100 IU/g; the other had no activity. Normal male breast contained 68-100 IU/g in 3 specimens and 1.7 IU/g in a fourth. Activity of normal male and female breast tissue averaged 66 $\pm$ 14 IU/g; it was significantly greater than that of breast cancer tissue ($p = 0.01$). Of 2 cases of gynecomastia induced by stilbestrol treatment of prostatic carcinoma, the tissue contained no activity in one and 1.3 IU/g in the other. Two bronchogenic carcinomas associated with hypercalcemia were assayed; neither contained detectible Vit. D-activity. Adipose tissue (abdominal panniculus) from 3 patients contained 3.1-15 IU/g. A single specimen of liver, assayed as a check on the method, contained more than 100 IU/g. In 6 instances, hexane extracts were prepared and contained the same activity as whole tissue. The only subject taking a Vit. D supplement, 800 IU daily, had no activity in her breast tissue.

While the presence of Vit. D-activity in breast cancer seemed to support the possibil-

ity that it might play a role in causing hypercalcemia, the quantitative aspects make this unlikely. Normal breast, which does not lyse bone when implanted adjacent to rat calvarium, contains more Vit. D-activity than cancer does. Disseminated breast cancer probably provides a greater volume of tissue, but assuming that 100,000 IU will usually invoke hypercalcemia in man, a tumor containing 5 IU/g would have to weigh 20 kg to produce this effect. Since secretion rates are unknown, this possibility cannot be entirely excluded.

Summary. To test the possibility that the hypercalcemia of human female breast cancer results from a Vit. D-like substance elaborated by the tumor, breast cancers, normal male and female breast and other tissues were assayed for Vit. D activity. Five of 6 cancers associated with hypercalcemia contained small amounts of activity; so did 3 tumors from normocalcemic women. The average activity of breast cancers was 15 $\pm$ 10 IU/g (mean $\pm$ SE). Normal male and female breast tissue contained 66 $\pm$ 14 IU/g. Two specimens of gynecomastia tissue and 2 bronchogenic carcinomas associated with hypercalcemia contained little or no activity. The small amount of Vit. D in breast cancer, a tissue which lyses bone, compared with the larger amount in normal breast tissue, which does not, makes it unlikely that Vit. D is responsible for the hypercalcemia of breast cancer.

1. Barnicot, N. A., *J. Anat.*, 1948, v82, 233; 1951, v85, 120.

2. Gordan, G. S., Eisenberg, E., Loken, H. F., Gardner, B., Hayashida, T., *Recent Progress in Hormone Research*, 1962, v18, 297.

3. Schachter, D., Kimberg, D. V., Schenker, H., *Am. J. Physiol.*, 1961, v200, 1263.

Received April 18, 1963. P.S.E.B.M., 1963, v113.