Serum and Tissue Calcium in Human Breast Carcinoma

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SUMMARY

Calcium determinations with atomic absorption spectrophotometry were performed on serum and on malignant tissues obtained from patients with carcinoma of the breast. No correlation was noted between either serum calcium levels or tissue calcium levels and the number of positive axillary lymph nodes. The tumor calcium content was greater than that of the control tissue. This latter finding is in conflict with some earlier reports concerning tissue calcium content.

INTRODUCTION

The calcium content of malignant tumors and the role of calcium in metastasis have been studied in the past (1, 3, 4). Fisher and Fisher (5) have recently reported on the calcium content of malignant tumors in rats and neither the serum calcium level nor the tumor calcium level appeared to influence the degree of metastasis. To the best of our knowledge, the calcium content of human breast cancer tissue has not been studied by atomic absorption spectrophotometry.

Utilizing this technique, we have attempted a pilot study of the relationship between serum calcium, malignant breast tissue calcium, and control breast tissue calcium. We have further attempted to see whether a correlation exists between these levels and the degree of metastasis noted in the axillary lymph nodes.

MATERIALS AND METHODS

This study includes 9 female patients with previously untreated breast carcinoma who were admitted to the Hospital of the University of Pennsylvania for surgical treatment.

Preoperative venous blood samples were drawn and the separated serum was stored at 4°C until analysis. Specimens of malignant breast tumors were obtained from each patient at the time of excisional biopsy. Following mastectomy, a second specimen was removed from the same breast at a site distant from the primary tumor; this “normal tissue” was used as the control. Information concerning metastasis to axillary lymph nodes and confirmation of malignancy of the primary tumor were obtained from the final pathology report.

The tissues, approximately 1 g/specimen, were homogenized in 5 ml 0.9% NaCl solution and were then centrifuged. Calcium concentration was estimated on the serum and supernatant material by atomic absorption spectrophotometry with a modification of the method of Willis (8). Standard calcium solutions were prepared from dried calcium carbonate in the range 2.0 to 20.0 mg/100 ml. Standards, serum, and supernatants were diluted 1:25 with 0.5% lanthanum chloride (with respect to the lanthanum ion), and the percentage of absorbance was recorded with the Perkin-Elmer Model 303 atomic absorption spectrophotometer. From a standard curve of the standards against percentage of transmission, the mg calcium/100 ml for each specimen were calculated. Each estimation was performed in duplicate and a set of standards was run with each set of samples. All tissue calcium values are expressed as mg/100 ml/g, wet weight, of tissues.

RESULTS

Of the 9 patients studied 7 had radical mastectomies and 2 had simple mastectomies. The serum calcium de-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>No. of positive nodes/total no. of nodes removed</th>
<th>Serum calcium (mg/100 ml)</th>
<th>Cancer tissue calcium (mg/100 ml/g wet weight)</th>
<th>Control tissue calcium (mg/100 ml/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/22</td>
<td>9.9</td>
<td>1.78</td>
<td>0.64</td>
</tr>
<tr>
<td>2</td>
<td>0/23</td>
<td>9.2</td>
<td>1.10</td>
<td>1.13</td>
</tr>
<tr>
<td>3</td>
<td>1/18</td>
<td>10.2</td>
<td>1.63</td>
<td>1.29</td>
</tr>
<tr>
<td>4</td>
<td>1/13</td>
<td>8.8</td>
<td>0.67</td>
<td>0.24</td>
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<tr>
<td>5</td>
<td>4/12</td>
<td>10.0</td>
<td>1.04</td>
<td>0.75</td>
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<tr>
<td>6</td>
<td>8/23</td>
<td>10.1</td>
<td>0.99</td>
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<tr>
<td>7</td>
<td>14/15</td>
<td>9.8</td>
<td>1.69</td>
<td>0.76</td>
</tr>
<tr>
<td>8</td>
<td>*</td>
<td>10.4</td>
<td>1.73</td>
<td>1.42</td>
</tr>
<tr>
<td>9</td>
<td>*</td>
<td>11.2</td>
<td>1.57</td>
<td>1.13</td>
</tr>
<tr>
<td>Mean*</td>
<td></td>
<td>1.36</td>
<td>0.93</td>
<td>0.41</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>0.41</td>
<td>0.37</td>
<td></td>
</tr>
</tbody>
</table>

* mg/100 ml/g, wet weight =
calcium concentration (mg/100 ml) in 5 ml tissue homogenate
wet weight homogenized tissue (g)
mg/100 ml/g, wet weight = mg/g, wet weight.

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mg/100 ml/g, wet weight × 5 = mg/100 g, wet weight.

1 Clinical Fellow, American Cancer Society.
2 Advanced Clinical Fellow, American Cancer Society.

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terminations ranged between 8.8 and 11.2 mg/100 ml for all patients (Table 1).

Eight of the 9 serum calcium values were within normal limits (i.e., 8.5 to 10.5 mg/100 ml). No correlation was noted between serum calcium levels and the number of positive axillary lymph nodes. Similarly, there was no correlation between serum and tumor levels of calcium.

The mean calcium value for malignant tissue was 1.4 ± 0.4 mg/100 ml/g, wet weight, and that of control tissue was 0.9 ± 0.4 mg/100 ml/g, wet weight. This difference noted between malignant and benign tissue from the same breast was found to be significant ($t = 3.3258, 0.01 < p < 0.02$). No linear correlation between the tissue calcium levels and the number of positive axillary nodes could be found.

**DISCUSSION**

Coman (2) in 1944 suggested that malignant cells possessed decreased adhesiveness and that this property might be due to a decreased calcium content. Subsequently, Brunschwig et al. (1) showed a slightly decreased calcium content in 14 gastric malignant tumors as compared to normal gastric mucosa. Dunham et al. (4) demonstrated a decreased calcium content in 11 cases of colon carcinoma, and deLong et al. (3) found the calcium content in intestinal tumors to be decreased in 12 cases.

We were unable to correlate our tissue values with our serum values and could correlate neither of these with metastasis. Of note is the fact that the cancerous breast tissues contained significantly more calcium than the control tissues. Hickie and Kalant (7), also using atomic absorption spectrophotometry, have demonstrated an increased calcium content of Morris hepatoma compared to normal rat liver. These increased calcium levels in both breast tissue and in rat liver are in conflict with the above-mentioned reports of decreased calcium levels in malignant tumors. In any comparison, however, the difference in methodology must be considered. Atomic absorption spectrophotometry has been shown to be accurate in the determination of trace elements in biological materials (6, 9). The method is especially advantageous at the low concentrations of the element such as were found in the tissue samples, errors arising from precipitation being avoided. The finding of an increased tumor calcium does not relate directly to Coman’s hypothesis concerning cellular adhesiveness because the total tumor calcium as measured above does not necessarily reflect the distribution of calcium ions at the periphery of the cell where adhesiveness is determined.

**ACKNOWLEDGMENTS**

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**REFERENCES**

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