PDR were immediately notified. Following this incident, all cytology brushes of this model in our stock were tested; one additional loose attachment was discovered. The design of this brush is questionable, but if "a needle is lost in a haystack," don't despair; it may be retrieved.

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A Rapid, Sensitive Method for Estrogen Receptor Analysis of Cells from Malignant Pleural Effusions in Recurrent Breast Cancer

To the Editor:

It has been shown that the presence of estrogen receptors in tumor tissue is related to a high response rate to endocrine therapy. McCarty et al. have demonstrated that malignant effusions in patients with recurrent breast cancer can be the source of tissue for estradiol binding analysis. We have developed a method for receptor determination by a whole cell binding analysis using a small number of cells in malignant pleural effusions from patients with recurrent breast cancer.

Three patients with recurrent breast cancer were studied. All had undergone radical mastectomy for breast cancer and were found to have pleuritis carcinomatosa on admission to Tokyo University Hospital. Cytologic examinations revealed that pleural fluids from these patients contained malignant cells. (3H)-Estriadiol binding analyses were carried out as follows: pleural fluid obtained from each patient was centrifuged at 4°C for 10 minutes at 450 x g. The supernatant was discarded, the pellet was obtained, and erythrocytes were lysed with lysing buffer which consisted of 0.155 M NH₄Cl, 10 mM KHCO₃, and 0.1 mM EDTA disodium. After erythrocytes were lysed, approximately 5-10 x 10⁷ cells were incubated for 90 minutes at 37°C under 5% CO₂ and 95% air in medium-199 containing (3H)-estradiol with or without unlabeled steroids. Bound (3H)-estradiol was recovered on glass microfiber filters. (3H)-Estriadiol bound to cells cannot be replaced by other steroids. Scatchard plot analysis of (3H)-estradiol binding to cells showed a single class of binding sites (Fig 1). For a control study, (3H)-estradiol binding was studied using cells obtained from pleural effusion of a 68-year-old man with hydrothorax which occurred in the nephrotic syndrome due to diabetic nephropathy. (3H)-Estradiol did not bind specifically to cells from this patient. These data are summarized in Table 1.

In this study, (3H)-estradiol binding was analyzed without purifying cells in malignant pleural effusion after erythrocytes were removed. Cells recovered contained approximately 10-27% of atypical cells. (3H)-Estradiol binding analysis by a whole cell competitive binding assay using cells from pleural fluids is a rapid and sensitive method. Results obtained would be an essential biochemical param-

![Graph](image_url)

**Figure 1.** (3H)-Estradiol binding to cells from malignant effusion after erythrocytes were removed (case 3). A. Binding vs (3H)-estradiol concentration. B. Scatchard plot analysis of specific binding.

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REFERENCES


<table>
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<th>Case</th>
<th>Age</th>
<th>Period after operation</th>
<th>Years after menopause</th>
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<th>fmol/10⁶ atypical cells</th>
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<tr>
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<td>10y</td>
<td>1</td>
<td>21.1 (27)</td>
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</table>

*Receptor concentration was calculated by Scatchard plot analysis.
†Cells obtained from pleural effusions after erythrocytes were removed.
‡Specific binding was proved; however, Scatchard plot analysis was not done due to insufficient cell number.
§Percentage of atypical cells.

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