clinical significance, which in recent studies, using intramammary injections of supravital dyes (*5*) and radiocolloid (*6*), has been shown to occur so infrequently that statistics are not recorded. That three out of seven patients (patients 110, 131, and 141) should demonstrate a phenomenon so rarely encountered would be an exceptional stroke of good fortune, beyond statistical and anatomic probability.

5. Contrary to statements dismissing radiocolloid lymphoscintigraphy as a technique "used mainly for demonstration of lymph drainage patency and normal location of lymph nodes," there is recent literature documenting the accuracy in distinguishing normal from abnormal lymphatics (*7,8*). Furthermore, there is also experimental evidence to suggest pathophysiological implications of radiocolloid deposition within lymph nodes, reflecting macrophage response to antigens (*9,10*).

6. In attempting to establish the validity of a new imaging technique, the authors should provide adequate detail of their computerized image processing. An objective discussion of both the amount of image manipulation and the signal-to-noise ratio is a minimal requirement, especially when some images involve a Tc-99m background-subtraction technique.

This arbitrary report of isolated observations carried out in uncontrolled circumstances has done a disservice to a novel and potentially valuable investigative approach. The data as collected and presented undermine confidence in the credibility of radioimmunodetection when few of the anecdotal cases recorded bear testimony to the target-specificity, sensitivity, or accuracy of imaging with radiolabeled anti-CEA antibodies.

A greater contribution to the principles embraced by scientific literature at large and to the elucidation of the pathophysiological mechanisms of a phenomenon of challenging dimensions, would have been derived from a more systematic approach:

1. Controlled studies in two groups of patients with comparable stages of breast carcinoma receiving I-131 CEA-AB and I-131 normal IgG in the hands and the feet would at least have provided evaluable data. Citing inguinal findings after I-131 normal IgG injection in a patient with ovarian carcinoma to support axillary findings after I-131 CEA-AB injection in a patient with breast carcinoma and palpable axillary adenopathy provides little assurance, let alone scientific evidence, for the foundation of a hypothesis.

2. Histological documentation of tissue changes at sites of radionuclide localization and radioautography of tissue sections would have contributed facts over and above conjecture.

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Reply

We appreciate the constructive comments and questions from Drs. Ege and Bronskill, since the information relative to their remarks may lend clarification where needed.

In a study, such as the one reported by us (*1*), several questions need to be answered before interpretations and deductive reasoning may be applied to the data:

1. Is the CEA antibody-CEA reaction in the lymph nodes specific?

2. If antibody is sequestered by lymph nodes in the absence of metastatic cancer, is the reaction focal, or is it systemic?

3. Will a nonspecific reaction occur between tumors and other immunoglobulins, such as normal IgG (goat)?

To prove that the antibody to carcinoembryonic antigen (CEA) will react with metastatic carcinoma, we chose the breast as the first model. Only patients with proven cancer of the breast were entered into the study. In only two of seven patients were the contents of the axillary region available for examination. In the other five patients the clinicians' level of confidence that the physical characteristics of the enlarged nodes in the axilla contained metastatic disease was such that excisional biopsy of the lymph nodes was not indicated or permitted. Subsequent physical examination of two of these five patients demonstrated continuing enlargement of "rock hard" axillary nodes. In none of the seven patients in this group was there evidence of other pathology or disease that may have contributed to the axillary lymphadenopathy. Portions of all the lymph nodes from the two radical mastectomies performed in this group were measured for radioactivity. In each case the lymph nodes with metastatic carcinoma demonstrated high levels of radioactivity in contrast to that found in the nonmetastatic nodes. We have observed the same results in two patients with metastases to inguinal lymph nodes from labial carcinoma, i.e., much higher levels of radioactivity in the lymph nodes that contained tumor (the involved lymph nodes were palpable in only one of these two patients). From our data no relationship of diagnostic significance could be established between concentration of radioactivity and palpability of nodes.

In three of the breast cancer patients, the CEA antibody was sequestered by the lymph nodes in the contralateral axilla. This incidence (43%) would be extraordinary in a large series of patients; however, in this study all three patients were inoperable and admitted to the hospital for chemotherapy. Clinically the surgeons were confident that the masses in the contralateral axillae of these three patients were unquestionably metastatic carcinoma. Contrary to the expressed impressions, the occurrence of metastatic breast carcinoma to the contralateral axilla is not that rare. Haagensen reported that his group clinically diagnosed contralateral axillary metastases in about 4% of their patients, and,

in addition, he states that R. S. Handley found contralateral metastases in 6% of 422 autopsies of patients with breast carcinoma (*2*). It could be postulated that such contralateral metastases were the result of a systemic pathway through the vascular system and then filtered out by the lymphatic system to be sequestered by the lymph nodes. Such a circuitous route, however, is far less probable than that a transverse connection of lymphatic channels to the opposite axilla exists.

Following the injection of Au-198 colloid into the areola and four quadrants of the breast, Vendrell-Torné et al. (*3*) found that migration of the colloid to the internal mammary lymph nodes from the lower inner quadrant was observed in 86% of his cases, from the upper inner in 62%, from the lower outer in 64%, and from the upper outer in 36%. These findings are very interesting in view of the far lower statistics reported for metastases to the internal mammary nodes. These investigators found that the radioactive colloid crossed the midline of the body in only two of their patients (0.8%), but they also recognized that the actual extent of drainage may not be recognized because of the low levels of radioactivity available for scanning. They also stated that their incidence of midline crossover of radioactivity was less than that reported by Rossi et al. (*4*). In a reported series of 100 normal internal mammary lymphoscintigrams, 5–6% of the patients demonstrated axillary sequestration of the radiocolloid. In this same series the authors found a 20% incidence of crossover from one internal mammary chain to the other. Based on their observation that the contralateral upper parasternal and supraclavicular nodes are visualized following an unilateral subcostal injection (in addition to the ipsilateral internal mammary nodes), they suggest that a cluster of sternomanubrial nodes may provide the communication across the midline of the chest (*5*). These data do indicate that our knowledge of lymphatic flow across the body midline is still quite incomplete.

It is important to establish whether the sequestration of CEA-antibody is a focal phenomenon or a systemic one, i.e., occurs randomly in any lymph nodal group of a patient with a CEA producing carcinoma. To obtain data that might clarify this point, patients were selected with proven carcinoma of organ systems whose lymphatic drainage channels would not usually pass through the axillary or inguinal nodal groups. Fifteen patients with genitourinary or gastrointestinal carcinoma were selected for this purpose. In all cases the primary carcinoma produced CEA. Of a total of 54 axillary and inguinal sites 45 were negative for sequestration of labeled antibody and nine (five cases) were positive (lymph nodes were not palpable in any of these patients). Based on the work of Nossal et al. (*6,7*), we postulated that sequestration of antibody in the five patients may be due to trapped antigen in lymph nodes that were in the drainage channels from either the primary or secondary tumors, particularly since surgery or recurrent carcinoma can change the usual regional drainage pattern. Since the publication of our article we have been advised that one of our patients (No. 189, 15 mo after anterior resection of sigmoidal carcinoma, Dukes' Type B) had an excision of the inguinal node in question 2 mo following our examination. Histopathologic examination showed no metastatic tumor, but did reveal germinal center hyperplasia. Two months after this procedure, his abdomen was explored and recurrent carcinoma was found in the pelvis. Although evidence of antigen trapping as the basis for an antibody-antigen reaction is circumstantial, Potomski et al. (*8*) demonstrated by immunofluoresence that carcinoembryonic antigen is trapped in regional lymph nodes (in the absence of metastases) that are in the drainage channels of carcinoma of the gastrointestinal tract. In contrast, they demonstrated that the immunofluorescence test was negative in lymph nodes outside of the drainage area. In studies of the type we have reported (*1*), histologic confirmation is frequently difficult if not impossible to obtain. It becomes necessary, therefore, to select patients who will provide the most satisfactory normal controls to provide an acceptable experimental design. This problem has been encountered previously in lymphoscintigraphy studies (*5*).

To date we have performed 23 whole-body scans on patients with proven carcinoma following the intravenous administration of normal goat IgG. In three cases, all large ovarian tumors, the neoplasms were defined on the images. In the surgical specimens, the I-131 tumor-to-nontumor ratios ranged from less than 1 to 1.1, suggesting that visualization of tumor was probably due to the vascular component only. These data have shown that IgG does not preferentially concentrate in CEA-producing tumors, whereas the CEA antibody does. In two of six patients (a total of 24 axillary and inguinal sites) concentration of activity was observed. In one patient activity was diffuse in the inguinal region following an orchidectomy and inguinal node dissection 2 wk before the radioactive antibody examination. Similar inguinal obstruction of labeled antibody has been observed in patients following vulvectomy and inguinal node dissection. With respect to the patient in whom the I-131 IgG concentrated at the site of a massive axillary metastasis, we only speculated that this might be based on obstruction.

It was not our intention to minimize the benefits of colloid lymphoscintigraphy, but rather to point out the difference in information obtained compared with that found with antibodies to CEA. In the context of target-specificity our statement that the results with colloid lymphoscintigraphy are nonspecific is accurate. Concentration of a colloid in lymph nodes as a sequestration by phagocytosis, which is a nonspecific process, has been established. Findings from colloid lymphoscintigraphy that suggest abnormal lymphatic channels or lymph nodes include: (a) absence or interruption of the chain of activity at one or more sites; (b) marked assymmetry in the size and/or density of the activity within the lymph node areas; (c) mottled or patchy appearance of the activity accumulation within the nodal regions; (d) presence of abnormal collateral lymphatic pathways; and (e) unusual enlargement of activity in the next group (*9*). Again each of these points may be an important factor in the interpretation of colloid studies, but none is "target specific."

In a study of the regional lymph nodes from radical mastectomies for carcinoma of the breast, Seaman and Power (*10*) found that normal lymph nodes may fail to concentrate the colloid, and, also, that lymph nodes with metastatic carcinoma may sequester the colloid appreciably. Perhaps the greatest specificity observed to date with Tc-99m sulfur colloid lymphoscintigraphy was the observation by Boak and Agwunobi that in seven patients with carcinoma of the breast and no ipilateral lymph node metastases, uptake of the colloid in the nodes was depressed (*11*). They found similar depression of colloid sequestration in the regional lymph nodes of animals with transplanted tumors (*12*). The authors speculate that this phenomenon may be due to changes in the lymph node from tumor products secreted in the lymphatic drainage. Could it be that this finding is the negative image of CEA-antibody trapped by sequestered CEA in the lymph nodes?

As referenced in our article (*1*) procedures for subtraction techniques have been described and explicit details of the procedure used for radioimmunodetection are in press (*13*). Since subcutaneous and intravenous labeled antibody were administered simultaneously, subtraction techniques were used *only* to provide more conspicuous images for photographic purposes. In practice, however, subtraction of background is not necessary for antibody lymphoscintigraphy; a major advantage over whole-body radiommununodetection.

We beg to differ with Drs. Ege and Bronskill that this study was uncontrolled with little evidence of target-specificity, sensitivity, or accuracy. In view of our whole-body studies with antibodies to tumor-associated antigens (approximately 270 patients), these

points have been well established (*14–16*).

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Sources of Error in the Calculation of Effective Renal Plasma Flow Using the Single-Injection, Single-Sample Technique

The comprehensive studies of renal function reported by Tauxe and Dubovsky (*1*) and others (*2–6*) utilize an open, two-compartment model to calculate effective renal plasma flow (ERPF) from a single injection of I-131 orthoiodohippurate (OIH). A blood sample is drawn at 44 min after injection and counted in a well counter. A diluted solution of the injected dose is also counted, so that the injected-dose counts may be compared with the counts remaining in the blood at 44 min. The ratio of injected-dose counts to plasma counts is proportional to ERPF, since a lower ratio of plasma counts to dose counts reflects greater clearance of OIH by the kidney. The ERPF is determined by a regression equation of the form $y = A + Bx + Cx^2$, where y is ERPF and x is the ratio of dose counts to plasma counts. The equation of best fit was determined by Tauxe et al (*7*) to be: $ERPF = -96.9 + 10.9x - 0.0454x^2$. This regression equation was based on an empirical correlation between I-131 OIH clearance and conventional *p*-aminohippurate clearance using the constant-infusion technique in 87 subjects. The correlation between the two clearance procedures was 0.95. However, the ratio of the injected dose to the plasma sample varied from a low of 15 to a high of 105 (Fig. 6 of Ref. *7*). These ratios correspond to ERPF values of 40 to 550 ml/min. In Fig. 1 the regression equation obtained by Tauxe et al. is plotted for ratios of injected dose to plasma sample up to and beyond 105. It will be seen that as the ratio of injected dose to plasma sample increases, the ERPF reaches a maximum at a ratio of about 120 and then declines, thus yielding a falsely low value. This fall off is due to the third term in the equation.

We have completed over 250 of these studies using the equation given above to determine ERPF. In general the test is repeatable and useful to our clinicians, but we have found that at least nine studies have had erroneous ERPF values due to the artifact inherent in the polynomial equation. In one patient the injected count was 284430, with 1260 counts in the plasma sample (consistent with a high plasma flow). This patient had BUN and creatinine clearances within normal limits. In addition, the shape of the time-activity curve indicated rapid uptake and good clearance in 30 min. The computation, however, shows an ERPF of only 48 ml/min; $x = 284430/1260 = 226$; $ERPF = -96.9 + 10.9(226) - 0.0454(51076) = 48$ ml/min.

Dubovsky (*8*) has recognized this problem and suggested a new equation for use with dose-to-plasma ratios above 120. However, no data were presented to demonstrate the accuracy of the new equation. The use of the present equation should be linked to the range of values used in the initial research. This can easily be accomplished by simply putting a ceiling on ERPF values. In this case a ratio of dose to plasma sample greater than 120 may be read as

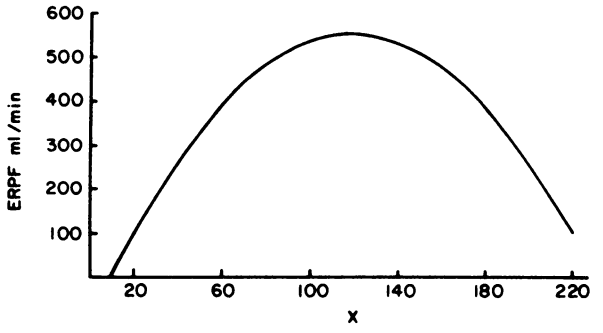

FIG. 1. Plot of regression equation $ERPF = -96.9 + 10.9x - 0.0454x^2$, where x = ratio of injected concentration of I-131 OIH to plasma concentration of I-131 OIH at 44 min. Equation was derived from values of x between 15 and 105 (*7*) and is valid for that range; it is invalid if $x > 120$.