LETTERS TO THE EDITOR

Re: Clinical Assessment of a Radioimmunoassay for Free Thyroxine using a Modified Tracer

We read with interest the letter by Drs. Bayer and McDougall (*1*) regarding Dr. Chan's evaluation of the single-step free-thyroxine (FT_4) assay marketed by Amersham Corporation (*2*). While we agree in general with their comments, there is one point that we feel we need to clarify, and a second upon which we wish to comment.

Our initial experience with Corning Medical's single-step FT_4 assay (Immophase Single-Step Free-T_4 (I-125) Radioimmunoassay) was presented at the meeting of the American Association for Clinical Chemistry in the summer of 1983. Information published in our abstract was included in Bayer's Table 1 (her Ref. *14*). Unfortunately, this represents information obtained with a prototype assay system and is not representative of the performance of Corning Medical's present single-step system, on which we report elsewhere (*4*). In 45 seriously nonthyroidally ill patients, 20% of results using the present Corning system fell below the lower limit for normal, while 42% of the same patients had results below the lower limit for normal using the Amersham FT_4 system (*4*). This difference reflects an awareness on the part of the Corning development staff that T_4 derivatives are bound by serum albumin. Their present assay system was modified after our original observations, such that these effects are minimized.

We agree that the Clinical Assays two-step system produces apparently normal results in patients who are nonthyroidally ill (*5*). We have, however, two concerns regarding this conclusion. First, we find that the antibody-extracted mass of total thyroxine is low in patients with nonthyroidal illness (*5,6*). If the mass extracted is quantified as extracted fraction times total T_4, the result is a low free-T_4 estimate. It is difficult to understand why this very same low extracted mass produces a quantitatively normal free-T_4 estimate in the Clinical Assays two-step system. Secondly, we, like Braverman (*7*), frequently find free-T_4 results that are apparently spuriously elevated when using the Clinical Assays two-step method. We have discussed this in some detail with Clinical Assays and have been informed that the problem does indeed exist and may or may not be explained by tubes into which serum is collected, as was suggested by Braverman (*7*). In our own laboratory we have repeated the experiment described by Braverman and his group, and have not been able to relate elevated FT_4 results to the brand of tube, whether the tube is glass or plastic, whether it is siliconized, or whether the red rubber top is used or not. Our inability to produce consistently reproducible results with this assay system is disturbing to us, because it certainly differs from the experience reported by the Stanford group (*1*).

In our experience, equilibrium dialysis most often produces normal FT_4 results in patients with nonthyroidal illness (*5*). In the absence of an equilibrium-dialysis assay, we most often successfully evaluate thyroid functional status in ill, hospitalized patients by measuring total thyroxine and T_3 uptake, followed by measurements of TSH and reverse T_3 concentration. We are unable to recommend any currently available commercial FT_4 system for FT_4 estimation in nonthyroidally ill patients.

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Reply

We thank Drs. Witherspoon and Shuler for their comments. We agree with them that the Clinical Assays two-step FT_4 procedure demands more technical skills from the analyst. As stated previously (Ref. *10* of our original letter), we have amended the kit protocol to obtain more reproducible results.

In agreement with Ekins et al. (*1*), we find it conceptually wrong to use any of the analog methods to measure FT_4, in particular in sick patients, if the tracer analog can be shown to bind to serum albumin or other serum binding proteins.

In view of earlier reports (*2*) and our own data, we believe it unusual that Drs. Witherspoon and Shuler find the measurement of total T_4 and T_3 uptake useful in ill, hospitalized patients.

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Re: Decreased Sensitivity of Early Imaging with In-111 Oxine-Labeled Leukocytes in Detection of Occult Infection

Datz et al., in their article in the March, 1984, issue of the

Journal, describe difficulty in obtaining early images using indium-111-labeled granulocytes to detect occult infection (*1*). In this study they used autologous granulocytes for labeling and imaging in patients with normal or elevated granulocyte counts. The early images, at 1-4 hr, had a sensitivity of only 33%. They therefore question our previous report of the rapid localization of activity to sites of infection, which were seen in granulocytopenic patients with known infections when given indium-111-labeled donor cells (*2*). However, I would like to reaffirm our observation of the rapidity with which labeled cells migrate in granulocytopenic patients. As a continuation of the previous report, studies done in nuclear medicine at our institution confirm this. The localization is clearly apparent, without computer manipulation of the image, as early as 30 min after injection of labeled donor cells.

I do not doubt that they are observing less localization at 1 hr in their autologous studies, but suggest that this difference is not a function of the technique, but is related to granulocyte kinetics and the differences in the marginating pool of granulocytes available in patients with a normal white-cell count contrasted with granulocytopenic patients. There may be a dilutional effect in patients with normal counts so that proportionally fewer labeled granulocytes migrate to sites of infection initially, because unlabeled granulocytes are also migrating there. In contrast, in granulocytopenic patients, the only circulating granulocytes are often the labeled donor cells, and they respond rapidly and in larger proportion to the chemotactic stimulus of an infection. This, in part, I believe explains the differences between these two studies.

Furthermore, in our study, we were imaging clinically apparent infections for purposes of evaluating transfusion response. It is possible that this involved a greater chemotactic stimulus than that in an occult, clinically nonlocalized infection.

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Reply

We thank Dr. Dutcher for her comments. Since we studied only patients with normal or elevated white counts whereas Dr. Dutcher's patients were granulocytopenic, a difference in leukocyte kinetics certainly could explain the disparity between her findings and ours.

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Re: Does Bone Measurement on the Radius Indicate Skeletal Status?

I read with interest the paper by Mazess et al. (*1*) and the statement that the "limbs . . . did not reflect the preferential osteopenia in the spine". I-125 absorptiometry of the distal third of the radius is chiefly a measurement of cortical bone, and thus a comparison has been made of cortical bone at one site with mainly trabecular bone in the spine.

The distal end of the radius contains significant amounts of trabecular bone and special-purpose I-125 CT scanners have been built that can measure the trabecular bone density very precisely (*2,3*). The distal radius is not only convenient and accessible for bone-density measurement but in osteoporotic patients is associated with fracture. In women approximately one third of all fractures occur at this site, and after age 55 the incidence of fracture in women is six times that in men (*4*).

For monitoring the course of osteopenia or its treatment, the method should have a reproducibility of greater than 1%, and there should be few obstacles to repeat measurements. We have built a low-dose CT scanner that uses an I-125 source (Hosie CJ, Richardson W, Gregory N, unpublished data). This is a self-contained unit, with image reconstruction carried out by a multiprocessor microcomputer. Trabecular bone density in the distal radius has been measured with a reproducibility of 0.5% in normal subjects and osteoporotic patients. Other groups have reported similar reproducibility with an I-125 computed tomograph (*2,3*) and have obtained good correlation between trabecular bone density of the distal radius and trabecular bone density of excised vertebrae (*2,5*). Our preliminary results indicate that in osteoporosis there is a preferential decrease of trabecular bone (45%) compared with that for cortical bone (30%).

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Reply

Hosie correctly notes that measurements of compact bone on the limbs do not reflect the trabecular bone of the axial skeleton, but suggests that measurement of trabecular bone of the distal radius may be clinically useful. Of course, absorptiometric scans on the distal radius are usually done at a site (10% of the forearm length) that is only about 10-15% trabecular, and even more distal sites are not more than 20-40% trabecular (*1*). We have found that shaft and distal sites on the radius are highly correlated (r = 0.95), and consequently absorptiometric scans at both locations must be equally poor indicators of spinal status (2). Computerized scanners based on x-rays and I-125 emission, such as those pioneered by the Zurich group cited by Hosie, provide precise measurements at the distal radius and other limb locations (proximal tibia). Rüegsegger (*3*) reported that trabecular bone of the distal radius was significantly diminished in osteoporotic patients. Nevertheless, there are two perplexing problems in addition to the high cost of these specially engineered systems. First, a technical difficulty is caused by the "environmental density" artifact (*4*). The trabecular bone on the distal radius (or tibia) is surrounded by a layer of much denser