to toxic nodules within the frame of Graves' disease that cannot. Furthermore, hyperthyroid patients with an increased ITI are more resistant to treatment (*3*). We also showed that a high IU (>40%) does not exclude iodine excess, nor does a normal urinary iodide, and the PB*I at 24 hr correlates negatively with ITI so that this measurement can be used to predict the iodide stores of the gland (*4*).

Of course, we are aware that the possibilities of measuring ITI are very scarce. Nevertheless, as we estimate that more than 30% of our hyperthyroid population present with iodine excess (especially the elderly as they need more radiodine), it is important to recognize some indicators that can be overlooked. Aside from the classical features, such as knowledge of iodine intake, a low IU and an increase of urinary iodide, the following factors should alert the physician to the possibility of iodine excess (at least in areas with relatively low iodine intake): a nonautoimmune toxic nodular goiter with a low PB*I at 24 hr and a suspiciously high T4/T3 ratio. Radioiodine treatment should then be adapted accordingly.

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REPLY: Your concurrence with the important differences between thyroid disease in Europe and the United States is of interest.

The points you make about the in intrathyroidal iodine pool are very well taken. Indeed I meant for this to be included as one of the many influences under the specified and unspecified "fudge factors" in Table 2.

X-ray fluorescent thyroid scanning to depict and quantify intrathyroidal iodide stores is an important and unique technique (providing data unavailable by RIU or urinary iodide measurements). My reason for not discussing the problems of variable intrathyroid iodide stores was primarily the space limitation for such editorials and the unfortunate fact that the technology of fluorescent thyroid scanning has not become widely disseminated and is not generally available in the U.S. (indeed no commercial devices are available). The alternative and much more readily performed study which is to determine the ^{131}I-PBI, as well as the ^{131}I-RIU, at 24 hr after tracer administration is an excellent suggestion and should be more widely practiced since it is within the ability of almost any laboratory with a wellcounter.

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Understanding Intracellular Metabolism: A Key to the Rational Design for Targeting Drugs and Radionuclides

TO THE EDITOR: I read with interest the article by Duncan and Welch on the intracellular metabolism of ^{111}In-DTPA-labeled receptor-targeted proteins (*1*). They demonstrate that in vitro these proteins are degraded in the lysosomes and that low molecular weight catabolites are formed containing the ^{111}In label. In follow-up studies, they have identified the metabolite as ^{111}In-DTPA-lysine. This metabolite remains in the lysosomes and is only slowly released from the cell. This may be one of the causes of the high renal and hepatic tracer accumulation as observed in scintigraphy of ^{111}In-labeled monoclonal antibodies.

This work shows an interesting relation with my studies on the targeting of drugs to the kidney and the liver using low molecular weight proteins and neoglycoproteins as carriers (*2–5*). In this concept of drug targeting, the lysosomal apparatus regenerates the active (parent) drug. In these studies, drugs were conjugated to proteins using a method similar to that applied by Duncan and Welch for ^{111}In-DTPA conjugation, i.e., an amide bond between the drug and the amino groups of proteins. My co-workers and I have demonstrated that lysosomal digestion of these conjugates results in drug-lysine compounds rather than the parent drugs (*2*). For instance, the anti-inflammatory agent naproxen conjugated to the low molecular weight protein lysozyme or to (neoglyco)albumins renders naproxen-lysine in lysosomal lysates. This is similar to the finding by Duncan and Welch that ^{111}In-DTPA-lysine is the major radioactive metabolite of lysosomal targeted proteins.

In contrast, as observed for ^{111}In-DTPA-lysine in vitro, naproxen-lysine is capable of leaving cells. This has been demonstrated for the lysosomal processing of naproxen-lysozyme and various naproxen-(neoglyco)albumin conjugates in vitro as well as in vivo (*3,4*). This suggests that derivatization of the ε-amino groups of lysines *per se* does not prevent lysosomal-efflux of lysine-derivatives; rather, other molecular characteristics such as size, charge and lipophilicity of the drug or nuclide-chelator complex may determine lysosomal efflux properties. Therefore, it might be interesting to learn whether ^{111}In-DTPA-COOH can be lysosomally regenerated in its parent form and whether it is capable of leaving lysosomes.

In targeting drugs with proteins, a spacer between the drug and the protein is essential to regenerate the coupled drugs in their parent form in the lysosomes. In this respect it will be interesting to investigate whether the lysosomal efflux can be modulated by using different coupling modalities to the protein. In principle, two different approaches may be of interest. One is the use of enzymatically degradable spacers, like L-lactic acid derivatives and the other is the use of acid-sensitive linkages. In the former case, DTPA can be indirectly attached to the protein via an ester bond. Ester bonds may be lysosomally cleaved by various carboxyesterases as demonstrated for naproxen coupled to proteins via alpha-hydroxy-acid spacers (*2,5*).

Due to the narrow specificity of lysosomal enzymes, the connecting bonds between drugs and amino acids often remain intact in lysosomes (*2*); therefore, an even more viable approach may be using acid-sensitive spacers between the chelator and the protein-backbone. One possibility may be the use of a *cis*-aconityl-like amide bond (*2*). In this concept the amide bond that is stable in the bloodstream is selectively cleaved in lysosomes due to the acid environment (pH = 4–5). The advantage of this approach above

the enzymatic approach is that in principle no enzymes are required to cleave these kinds of bonds.

I share the authors' view that intracellular metabolism can very well be studied in in vitro model systems; however, it should be noted that in vitro procedures may suffer from limitations like decreased enzyme activity of cell-homogenates and lysosomal lysates. These can be caused by isolation artefacts, like loss of co-factors during the work-up of these preparations. Therefore, a combination of in vitro and in vivo studies are needed to obtain optimal results on the intracellular metabolism of receptor-targeted proteins. Finally, the identification of metabolites like ^{111}In-DTPA-lysine contributes to the understanding why high background radioactivity of labeled monoclonal antibodies is observed in the liver and kidneys. Improved knowledge on this point aids in the rational design of improved drug/nuclide targeting preparations.

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REPLY: We appreciate Dr. Franssen's interest and insightful comments. We agree entirely with the central theme of his letter: an understanding of intracellular metabolism will be key in the rational design of targeted drugs and diagnostic agents. We also agree with his contention that derivatization of the ϵ-amino group of lysine does not prevent lysosomal efflux of lysine derivatives per se. Rather, we suggested that ^{111}In-DTPA-amino acids would remain within the lysosome since their positive and negative charges would limit diffusion across the membrane (*1*). We also believe that size, charge and lipophilicity of the drug or nuclide-chelator will determine lysosomal efflux properties. When identifying in vivo metabolites, we found the predominant metabolite was ^{111}In-DTPA-ϵ-lysine and only small amounts of ^{111}In-DTPA were produced (*2*). We were pleased to find Dr. Franssen's work, as well as other reports that suggest that lysosomes infrequently hydrolyze the amide bonds between lysine and "foreign" substances such as drugs and chelates.

We also hope to modulate the rates of lysosomal efflux through appropriate choices of chelates and their linkages to protein backbones. We plan to test these using reconstituted systems, cell culture models and in vivo experiments.

In summary, we hope all our correspondence with this journal will be in the same tone of complete agreement.

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TBI Is Not MIBI

TO THE EDITOR: I read with great interest the case report by Desai and Yuille describing ^{99m}Tc-MIBI uptake in a recurrent carcinoid tumor (*1*). Three ^{99m}Tc-labeled isonitriles have been evaluated in detail in humans: TBI (tertiarybutyl isonitrile), CPI (carbomethoxyl isopropyl isonitrile) and MIBI (methoxy isobutyl isonitrile) (*2–4*). High uptake in the lung and liver limited the clinical use of ^{99m}Tc-TBI (*2,5–7*). However, ^{99m}Tc-MIBI, an ether-substituted analog of ^{99m}Tc-TBI, has gained wide clinical acceptance.

Desai and Yuille stated that Ramanathan et al. (*8*) had used ^{99m}Tc-MIBI for visualization of suppressed thyroid tissue, confusing TBI with MIBI. In fact, Ramanathan et al. have used ^{99m}Tc-TBI for this purpose. Although both ^{99m}Tc-MIBI and ^{99m}Tc-TBI are essentially isonitriles, they have different chemical structures, biological behavior and in vivo distribution (*2,5–11*).

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