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Reply

Dr. Selikson's comments are appropriate in stressing the well-known fact that the FDG rate constants may change unpredictably, not only from patient to patient, but also from one brain region to another within the same patient, particularly in pathologic conditions. The effects of such changes on the calculated GlMR have been the subject of theoretical (*1-2*) as well as patient (*3*) studies. The results of these studies demonstrated that large changes in rate constants could result in relatively marked errors on calculated GlMR. In our paper (*4*), we pointed out that the GlMR values found in stroke patients using the in vivo autoradiographic approach of Phelps et al. (*5*) (which includes k_4, the FDG-6P dephosphorylation rate constant) should be interpreted with caution, a fact stressed earlier by Kuhl et al. (*6*). We also indicated that more accurate GlMR measurement could be obtained if kinetic analysis of the F-18 cerebral accumulation curve is performed, a procedure by which the FDG rate constants can be estimated regionally. We are currently analyzing the data in this way and comparing in the same patients the GlMR values measured by both methods in order to establish further the validity of the autoradiographic approach using adapted (e.g., "ischemic") rate constants. We therefore agree with Dr. Selikson's suggestion that more work is needed if reliable measurement of regional GlMR in pathologic conditions is desired.

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Reply

No one can argue with the fact that deviations in the dynamic rate constants for the transport of deoxyglucose will affect values of glucose metabolic rate (GMR) as determined by the Sokoloff method. However, the situation is not as black as Dr. Selikson indicates. For one thing, he failed to point out that the simplified formula presented in his Ref. 4 reduces the error caused by these deviations, and this reduction ranges from 10% to 50% for the two examples cited. Another fact not mentioned is that, by doing patient scanning during the dynamic uptake period, one can completely eliminate the effect of the deviations (*1*).

It is not correct that the cerebral glioma project at our institute did not include a rigorous monitoring of the rate constants. As mentioned in the article (*2*), and as reported in more detail at the American Neurological Association meeting (*1*), we have measured these constants in gliomas by dynamic scanning and found them to be relatively unchanged.

Finally, one should be very cautious about recommending the abandonment of noninvasive clinical measurements because their accuracy is in question. It is often difficult in medicine to measure basic quantities with absolute accuracy. The point is that the quantity measured, even if it includes "contaminating" effects, may be of great clinical value. This has proven to be the case in our glioma study, where any shifts in rate constants and lumped constant (which may be of even more concern) have not prevented this method from becoming the most successful technique for diagnostic grading of these lesions.

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Re: Indium-111 Tropolone Versus Oxine

We should very much like to support the comments of Dr. Goedeman (*1*) on the following subjects.

1. The solubility of oxine in aqueous acidic solutions is good, and crystalline oxine may be dissolved directly in concentrated acid at concentrations of 100 mg/ml, thus avoiding the need for ethyl alcohol as a solvent. This is apparently the case for both commercially available European oxinates (*2,3*).

2. The comments regarding the labeling efficiency with tropolonate (*4*), subsequently supported by the work of Danpure et al. (*5*), show that in plasma, cells can be labeled only if concentrated five- to tenfold, and the labeling efficiency is then only 50-70%. In contrast, the indium-binding ligands, oxine, acetylacetone, and tropolone, will all, in the absence of plasma and in physiological cell concentrations, give almost quantitative labeling efficiency in very short labeling times of less than 2 min (*6*). This is the case for both platelets and leucocytes of common laboratory animals and man.

3. It is dangerous to extrapolate platelet labeling data obtained from canine experiments, since canine plasma is less effective than human plasma at inhibiting indium-oxine uptake. This is probably due to a decreased concentration of lipoproteins in the dog (*7*).

We stress further that in the search for the ideal cell-labeling method, cell function in vivo must be retained at all costs. This can

be achieved only by minimal handling, minimal addition, and minimal ex-vivo time. The concept of labeling in plasma is a step in the right direction, but if cell concentration should be necessary the advantage is lost, especially if labeling efficiency is also reduced. We feel that it is not sufficient to quote biological handbook data based on LD 50 (*8*) but to plot ligand concentration against cell damage and labelling efficiency in order to select the best agents.

We believe that, of the three simple ligands currently reported, oxine is probably the most suitable and least toxic for normal cell labeling. The concentration of acetylacetone, combined with the very narrow concentration range to achieve high labelling efficiencies, limit its usefulness (*9*). Tropolone at optimal labeling concentrations in plasma can be shown to cause structural changes in cells (*10*).

We should like to see our commercial colleagues concentrating not on new nonspecific labeling agents, but upon cell-specific agents or on complete laboratory kits for the smaller hospital, thus making the cell-labeling technique more readily available and reproducible between research and diagnostic establishments.

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Reply

I find the comments of Dr. R. J. Hawker interesting. Some have been substantiated however, others are difficult to prove.

We have reservations concerning their remarks about the solubility of oxine in aqueous solutions. Oxine is soluble in acid solution, but we are interested in oxine at pH 6.5–7.4 for cell labeling. Without any proper solvent, oxine and In-oxine will be in the colloidal form. In nonphagocyte cell labeling, it is important to add alcohol and make In-111 oxine soluble. It is difficult to check colloid sizing with filter papers of different porosity. Most of the neutral In-111 complexes are very sticky and spread on millipore surfaces and our preliminary studies on In-111 oxine colloid sizing using the scanning electron microscope were not helpful either, though In-111 oxine is available without alcohol, this does not necessarily mean that In-111 oxine is water soluble. The true physical form of In-111 oxine is irrelevant as long as cell labeling can be performed uniformly and consistently. The uniform distribution of In-111 on a cell population might be more in doubt if In-111 oxine colloid were used. Since we are quantifying platelet thrombosis and expressing the interaction of platelets with a de-endothelialized vessel wall in terms of platelet density per unit surface area of artery, vein, or synthetic vascular graft (*1*,*2*), this uniformity is critical.

We consistently found 15–30% higher platelet (human) labeling efficiency with In-111 tropolone than with In-111 oxine. Platelet extraction in an ACD-plasma medium was never 100% with any of the ligands, tropolone, oxine, or acetylacetone (*3–6*), and labeling efficiency with human platelets is 5–10% lower than with canine platelets under identical conditions of cell number and ACD-plasma volume. Moreover, we do not like to use PGE_1 in incubation media. Drs. Lavender and Subramanian considered In-111 tropolone as the preferable label for platelets and white cells (*8*), although Thakur suggested that In-111 mercaptopyridine oxide might serve the same purpose equally well (*9*).

Partitioning of In-111 complexes was quantified by lipoproteins of dog and human plasma and no marked difference was observed. Lipoproteins do not extract In-111 complexes as efficiently as cell membranes. Lipoprotein binding of these three In-111 complexes in human plasma was studied by cesium chloride density-gradient separation and ultracentrifugation. Human plasma (18 ml) was incubated with 50 μCi each of In-111 oxine, In-111 acetylacetone, In-111 tropolone, I-131 iodocholesterol, and C-14 cholesterol. The fractions of very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), albumin, and gamma globulin (A + GG) are separated by spinning 4.5 ml at 210,000 *g* for 12, 16, and 20 hr using density adjustment with cesium chloride solution at 1.06 and 1.25 g/ml respectively. The radioactivity in four fractions was determined in a gamma well and liquid scintillation counter, and the percentage of activity with different lipoprotein fractions is quantitated and tabulated below.

	VLDL	LDL	HDL	A + GG
In-111 Tropolone	4.20	9.58	29.90	56.29
In-111 Oxine	6.63	14.91	26.45	52.00
In-111 Acetylacetone	4.99	24.99	40.00	28.33
C-14 Cholesterol	23.83	63.40	11.80	2.33
I-131 Cholesterol	28.69	55.25	10.23	5.84

In cell labeling with microgram amounts of oxine and tropolone, toxicity is only of theoretical interest, (*7*) since most of these ligands wash out during cell labeling. Of the three ligands, In-111 $(tropolonate)_3$ appears to be the best agent at this time.

After reviewing hundreds of transmission and scanning electron micrographs of platelets, polymorphonuclear leukocytes, and endothelial and smooth-muscle cells, we are convinced that definite conclusions need careful evaluation of a statistically significant number of cells by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The artifacts in micrographs must be properly scrutinized. Many changes in cells labeled with In-111 oxine/tropolone are observed by SEM or