the correlation of Creese et al. implies that flow is not the major determinant at pharmacologic doses.

In the May issue of the *Journal* there are two papers and several abstracts on receptor/binding radiotracers (*2,10,11*). One such abstract (*12*) suggested receptor-mediated localization of C-11 practolol despite a low affinity constant. Many ligands thought to act in vivo through a receptor mechanism have been radiolabeled. Although claims for receptor-mediated distribution of radioactivity have been made, few of the radiotracers have been validated by applying the operational definition of a receptor-binding ligand.

What is the most convincing experiment, given that receptors are usually identified by an operational definition because few receptors have been characterized chemically? Kahn defines a receptor by its binding properties (*13*): (a) binding to the receptor is rapid and usually reversible; (b) there is a finite number of receptor sites on the cell; (c) receptors have a high affinity for the ligand; (d) binding sites are specific for the ligand and the binding sites can be related to biologic (or pharmacologic) effects of the ligand. Zanzonico et al. have used Criterion (b) to validate F-18 haloperidol as a receptor-binding radiotracer. Proof of saturability is given, but the pharmacologic specificity (Criterion d) as demonstrated by Creese et al. argues against haloperidol's being distributed by a receptor mechanism with higher specificity than spiroperidol. In general, saturability appears to be better proof of receptor binding than pharmacologic specificity, because the effect of specific activity has to be evaluated very carefully in the latter. But use of a single criterion, as by Zanzonico et al., can lead to false conclusions because of the complicated system and the chance of technical error. Validation using at least two criteria would seem more prudent.

One of the most powerful proofs for receptor binding is stereoselectivity. If two isomers are available, one with pharmacologic activity and one without, then a clear experiment is evident. Arnett et al. (*14*) have used this to validate receptor binding for C-11 spiroperidol. In their case (+) butaclamol displaced C-11 spiroperidol, whereas (−)butaclamol did not. The relative displacing power of the (+)butaclamol for haloperidol and spiroperidol would be a firm indication of the relative receptor binding of each. We have also used (R)-QNB and (S)-QNB to validate the receptor binding of tritium-labeled (R)-QNB in vivo (*7*). These studies minimize the effect of the large dose of competitor on blood flow, transport, and metabolism.

Another strong proof of the relative receptor binding of two radioligands is the use of a series of nonradioactive receptor-binding ligands. Chang and Synder (15) have determined in vivo ID_{50} values by injecting dose ranges of various compounds known to bind to the benzodiazepine receptor. Their major evidence for receptor binding is the pharmacologic specificity of the binding sites. Likewise, H-3 QNB has been shown to bind to the muscarinic cholinergic receptor, by demonstrating, using various antagonists, that the pharmacologic profile in vivo is identical to that obtained in vitro (*7,15*). These can easily be applied to two radioligands to determine which has the higher receptor specificity (*16*). The major advantage of the stereoselectivity study and the in vivo pharmacologic profile is relative abundance of evidence to support a claim of receptor binding. A single specific-activity study does not provide such support. In addition to the call for stable tritium-labeled radiotracers, perhaps the *Journal* should add the need for validation by at least two criteria in the definition of a receptor.

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Reply

Drs. Eckelman and Gibson raise a number of interesting points about the analysis of the difference between the H-3 and F-18 haloperidol distribution data (*1,2*). However, the editorial covered two points, first the specific differences between the tritiated and fluorinated haloperidol, and second the general potential pitfalls in obtaining receptor-density data from in vivo studies of labeled ligand uptake.

Given that the H-3-labeled haloperidol loses most or all of its tritium (*1*), then there is no need to look for any other explanation

of the difference in distribution between the H-3 and the F-18 data, since this is entirely sufficient. The use of pharmacological data to query the tracer data is probably not appropriate in this case. We have shown (*3*) that the percentage in brain uptake of haloperidol decreases as the delivered dose of haloperidol increases, from a tracer quantity to a pharmacological dose. Although these data refer only to gross uptake and not to specific receptor binding, the change in percentage uptake as a function of total concentration would be expected to produce a change in the percentage bound to the receptor. Of much greater concern is the fact that another study (*4*), using flucrine-18 haloperidol of much higher specific activity, gave biodistribution data in good agreement with the H-3 data and apparently at odds with the data obtained by Zanzonico et al. (*1*).

The much higher counting rates obtained by the use of material with high specific activity substantially simplifies the experiments and reduces the random errors inherent in the technique. Zanzonico et al. (*1*) gave very few details of the techniques used to obtain accurate results with the extremely low counting rates that they encountered. The two sets of data were obtained in different animals (mice and rats, respectively), and species differences may account for the different results. In the absence of any correlation experiments between the two species used, however, the much lower random errors encountered in the high-specific-activity study must give greater confidence in those data.

As to the more general points concerning the quantitative distribution of the radioligand as a function of receptor density, Drs. Eckelman and Gibson are quite correct in pointing out that Klotz's criticism (*5*) of the Scatchard analysis applies only to a multicomponent binding curve. With the use of H-3 ligands and conventional techniques, however, any component with higher association constants but more than an order of magnitude lower capacity will be extremely difficult to detect. With the much higher specific activities possible with other radionuclides, such components may well reveal themselves in unexpected fashions. At the St. Louis meeting last year, Dr. Friedman (*6*) reported just such a component in the work with [^{75}Br]bromospiroperidol. Other explanations for differing distribution with different specific activities—even when these are well below receptor-saturation levels—are also possible. The presence of endogenous ligand for the receptor may well play a key role in the quantitative binding, as has been shown with bromospiroperidol.

The results obtained with (R) and (S) QNB demonstrate that the uptake is not solely a function of flow but is receptor mediated. These results, however, do not establish that flow has no effect on the receptor-mediated uptake. The critical question is whether areas of tissue with the same receptor density but different blood flow will accumulate the same amount of ligand. The hepatic results of Krohn et al. (*7*) suggest that the answer to this question may be a function of the properties of the labeled ligand, even when all the other criteria for ligand binding have been met.

In vivo demonstration of true receptor binding is a major undertaking, and it is true that a saturation curve, or differential binding with increasing amounts of ligand, is a necessary but not sufficient condition to demonstrate that receptor-mediated binding is occurring. The dynamic nature of the in vivo process implies that the rates of the different processes involved in the receptor-mediated binding bear as important a role as the absolute values. As these rates are largely unknown at present, the full implications of all these data are difficult to assess.

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Reply

In general, the validation in vivo of a putative receptor-binding radiotracer as such is complicated by pharmacokinetic, metabolic, and pharmacological considerations. As alluded to by Frost and Kuhar (*1*), it is this complexity that makes rigorous application of the operational definition of a receptor-ligand interaction (*2-4*) difficult in vivo. Consequently, the development of a putative receptor-binding radiotracer is initiated, logically, on the basis of in vitro experimental data (*1*). The interaction of haloperidol with the dopamine receptor, in particular, has been extensively validated and characterized in vitro, with rigorous application of the operational definition of a receptor-ligand interaction (*5,6*). It is on this basis that we initiated our development of F-18 haloperidol as a radiotracer binding to the dopamine receptor. It is on this basis, also, that our in vivo experimental data were interpreted.

The objective of our study, therefore, was not to validate exhaustively the interaction between haloperidol and dopamine receptor—since many studies concerning such validation in vitro (*5,7*) and in vivo (*7-9*) have already been published—but to evaluate F-18 haloperidol further for in vivo use as a radiotracer binding to the dopamine receptor. As we state in our paper (*10*), "the dose-dependent decrease, in the relative concentration in the striatum and in the striatum-to-cerebellum concentration ratio, is consistent with receptor-mediated localization of F-18 haloperidol in the striatum." Certainly, our findings do not establish conclusively that striatal localization of F-18 haloperidol is receptor mediated. Indeed, based on the saturable nature of cerebellar localization of F-18 haloperidol, some portion of its dose-dependent striatal relative concentration may actually reflect saturable blood-to-brain transport (i.e., carrier-mediated transport) of haloperidol (*10*).

As we explicitly stated, further evaluation of F-18 haloperidol as a radiotracer binding in vivo to the dopamine receptor will entail application of additional criteria for a receptor-ligand interaction, together with further intercomparison with spiroperidol and other dopamine-receptor-binding radiotracers. Certainly we should pursue the application to F-18 haloperidol of the elegant method applied by Arnett et al. (*11*) and Laduron et al. (*12*), e.g., the use of receptor-binding and nonreceptor-binding stereoisomers to discriminate between specifically- and nonspecifically bound ligand in tissue. Contrary to the assertion of Eckelman and Gibson, however, implicit in our experiments is the application of at least