sidered. We note that the results shown in Johnson's and Dunford's Table 1 agree exactly with the MIRD value if the marrow and red blood cell (RBC) values are added, except for the slight difference when excretion is assumed. Putting activity into the RBC compartment also introduces changes in the residence times in organs, particularly in the spleen.

Some of the other points depend upon whether modeling is to be used to achieve an estimate of radiation dose or to simulate the physiological aspects of iron metabolism. For radiation-dose calculations, continuous infusion and single injections give the same results, but the amounts present at a given time in a particular compartment or organ are different. The use of the 120-day life for RBCs is a reasonable assumption for radiation dose calculations, but in reality there is an appreciable range from 100 to 135 days, as noted in the MIRD report. Even in an individual the spread in RBC lifetimes results in a progressive smearing out of the spectrum of the times at which iron is released from the RBCs and is reutilized. The difference between the assumptions made for the dose calculations and reality after 400 days could be significant. We realized that by continuing the 120-day recycling after 400 days, an answer would be obtained different from that obtained by assuming exponential decay thereafter, but we question whether such a refinement gives a more reliable estimate of the real absorbed dose. As Johnson and Dunford point out, by this time the effect of excretion will have become significant for Fe-55, and this is probably more important than the other suggested refinements of the calculation.

We feel that, by possibly erring on the high side in the dose estimates for the bone marrow, we are being conservative and that if these estimates are used for restricting the amount of activity administered to patients, the risk to the patients is minimized. Absolute accuracy is a desirable goal, but it is unrealistic in the presence of wide individual variations in the kinetics of iron metabolism. The model has been kept sufficiently simple to be used in the relatively small computers available in nuclear medicine laboratories while preserving at least the main features of iron metabolism that are significant in calculations of the absorbed dose.

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1. Radiation absorbed doses from iron-52, iron-55, and iron-59 used to study ferrokinetics. No 11: MIRD Dose-Estimate Report J Nucl Med 24:339–348, 1983

Re: Receptor Binding of F-18 Haloperidol and Spiroperidol

In a recent editorial Dr. Timothy Tewson (1) gave three possibilities for the inconsistencies in the distribution of H-3 haloperidol and F-18 haloperidol as described by Zanzonico et al. (2). These can be outlined briefly as: (a) artifacts from using a labile tritium-labeled compound; (b) the difference in distribution between ligands of high and low specific activity; and (c) flow-limited distribution. This analysis raises a number of important points.

Zanzonico et al. have used their F-18 distribution data (Table 1 of Ref. 2) and the dose dependency indicating receptor binding (Fig. 2 of Ref. 2) to suggest that F-18 haloperidol is superior to spiroperidol because the absolute concentrations are higher

whereas the striatum-to-cerebellum ratio is similar (Table 1 of Ref. 2). Creese et al. (3) however, have shown that for a great number of drugs there is a correlation between the pharmacologic effect of a drug and the receptor affinity determined in in vitro experiments using isolated tissue. This recent Science article showed that spiroperidol has a higher pharmacologic effect and a higher in vitro affinity constant relative to haloperidol. The use of an extreme example (domeridone) by Zanzonico et al. does not nullify the correlation of Creese et al. Since Zanzonico et al. have shown that H-3 haloperidol resists detritiation in in vitro assay systems, the determination by Creese et al. should be accurate. But the F-18 haloperidol distribution data (Table 1 of Ref. 2) do not agree with the pharmacologic data. The key word in the Creese correlation is "pharmacologic." The average clinical dose for spiroperidol is 58 nmol/kg, and for haloperidol 152 nmol/kg (3). Zanzonico et al. used doses from 0.01 to 100 μg/kg (or 0.02 to 250 nmol/kg), the latter clearly in the range of the pharmacologic dose. Therefore, there should be a correlation between the pharmacologic effect and the affinity constant, and a correlation between the affinity constant and receptor occupancy (radioligand distribution) by the laws of mass action. Since receptor occupancy is related to pharmacologic action, then either Creese's correlation is incorrect or the F-18 haloperidol distribution data of Zanzonico et al. are incorrect. The fact that H-3 haoloperidol is rapidly metabolized is an important observation, but it may not be relevant to the argument that F-18 haloperidol as prepared by Zanzonico et al. gives the actual haloperidol distribution. Even if haloperidol did not release tritium, it may still not show receptor binding in vivo.

Nevertheless, proof of receptor binding based on the operational definition of biological or pharmacologic specificity is difficult, because of the effect that specific activity can have on the distribution. Krohn et al. (4) did indicate that ligands of high specific activity may not distinguish between different receptor concentrations, as Tewson stated. But Krohn's argument is based on the fact that at "low-receptor-occupancy . . . pharmacologically active ligands . . . will not distinguish receptor population from rate-constant effects. Receptor mapping will be accomplished only if the binding rate constant is pathologically invariant, a situation that should not be assumed without thorough testing in vivo (4)." This argument is not relevant to the present case, and cannot be used to explain the higher receptor binding of haloperidol relative to spiroperidol, as reported by Zanzonico. These results are also unrelated to the paper by Klotz, who is dealing with the analysis of multicomponent curves (5,6). Klotz discussed the difficulty in the proposed methods of analyzing in vitro binding data to provide (a) the number of components, (b) the respective affinities of each component for the radioligand, and (c) the biological relevancy of each component. He likewise was not commenting on the determination of the receptor concentration using radioligands of different specific activity. The more relevant discussion of the effect of specific activity has been put forth by us to explain attempts at in vivo determinations of receptor density (4,8). No in vivo data have been put forward as a determination of receptor density; rather receptor density has been determined by in vitro tests using isolated tissue. (Recently Mintun et al. have presented a model to calculate receptor concentration in the dopamine system (9).) We, in fact, have argued that these in vivo experiments attempting to prove agreement between in vitro and in vivo data actually achieved only apparent agreement because of the specific activity used. The coincidental "self-fulfilling prophecy" has been disproven for H-3 QNB. At various specific activities, various striatum-to-cerebellum ratios are obtained. Even the maximum value underestimates the relative muscarinic-receptor densities found in those two structures.

The final argument also attributed to Krohn et al. (4) does not seem to apply in the case of these agents in the brain. If they are measuring blood flow, the uptake values would be the same. Again

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