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.

TIMOTHY TEWSON
University of Texas Health Sci. Ctr.
Houston, Texas

REFERENCES

1. ZANZONICO PB, BIGLER RE, SCHMALL B: Neuroleptic binding sites: specific labeling in mice with [^{18}F] haloperidol, a potential tracer for positron emission tomography. *J Nucl Med* 24:408-416, 1983
2. TEWSON TJ: Radiopharmaceuticals for receptor imaging teaching editorial. *J Nucl Med* 24:442-443, 1983
3. TEWSON TJ, WELCH MJ, RAICHLE ME: Preliminary studies with ^{18}F-haloperidol. A radioligand for in vivo studies of the dopamine receptor. *Brain Res* 192:291-295, 1980
4. WELCH MJ, KILBOURN MR, MATHIAS CJ, MINTUN MA, RAICHLE ME: Comparison in animal models of ^{18}F-spiroperidol and ^{18}F-haloperidol: Potential agents for imaging the dopamine receptor. *Life Sci*:in press
5. KLOTZ IM: Number of receptor sites from Scatchard graphs: facts and fantasies. *Science* 217:1247-1249, 1982
6. DEJESUS OT, REVENAUGH J, DINERSTEIN R, et al: Measurement of dopamine receptor densities. *J Nucl Med* 24:P70, 1983 (abst)
7. KROHN KA, VERA DR, STADALUIK RC: A complementary radiopharmaceutical and mathematical model for quantitating hepatic-building protein receptors. In *Receptor Binding Radiotracers*. Vol. II, Eckelman WC, ed. Boca Raton, FL, CRC Press, 1982, pp 41-60

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

three criteria [saturability, anatomic specificity, and recoverability (10)], not one, for a receptor-ligand interaction (2-4). We note that in their recent paper in *Science*, other authors (13) have applied two (i.e., saturability, anatomic specificity) of the three criteria (10), and on that basis concluded that dopamine receptors in the human brain had in fact been imaged by positron tomography. With regard to the recommendation that at least two criteria be applied to the validation in vivo of receptor-binding radiotracers, we feel that in light of the current, rather germinal stage of development of such radiotracers, it is still too early to establish rigid rules for such validation—e.g., concerning the number or precise nature of criteria to be applied.

An apparent discrepancy between the results of Creese et al. (14) and Zanzonico et al. (10), was cited by Eckelman and Gibson as justification for stating that the data of either the one group or the other are incorrect, and that possible errors in the F-18 haloperidol data of Zanzonico et al. (10) may explain this discrepancy. If any reader of a scientific journal believes that a published study is flawed by one or more technical errors, it is our opinion that he should provide evidence for such an assertion. We are confident of our results, but at the same time are constantly striving for accuracy and scientific rigor. Consequently, we would welcome any specific suggestions directed toward the improvement of our experimental method.

Nevertheless, we do feel a discussion of the consistency of our findings relative to available pharmacologic data is pertinent, and we welcome the opportunity to respond to this point. The correlation analysis of Creese et al. (14) is based upon the average daily dose (normalized to body mass, and generally long-term) of neuroleptics used in the management of schizophrenia. Due to differential pharmacokinetics and bioavailability (e.g., due to blood-brain barrier penetrability) among drugs, this analysis would be greatly refined if it were based upon the time-averaged target tissue (i.e., striatal) concentration of neuroleptics. This is because the mean fractional receptor occupancy—and presumably the net pharmacologic effect—depend upon both the receptor-binding affinity and the mean of the time-dependent drug concentration at the pharmacologic site of action. The assertion by Eckelman and Gibson that our findings are inconsistent with those of Creese et al. (14) is, therefore, difficult to rationalize because it implicitly assumes that, for haloperidol and spiroperidol, the concentration in mouse striatum at a single time point (i.e., 1 hr) after one intravenous administration accurately approximates the concentration in human striatum at steady state after long-term daily oral administration, which in turn is assumed to be directly related to the daily dose normalized to body mass. Interestingly enough, if one tentatively accepts the validity of this assumption, we can demonstrate (see below)—contrary to the claim of Eckelman and Gibson—that our findings are, in fact, reasonably consistent with those of Creese et al. (14).

In evaluating the psychotropic potency of a large series of neuroleptics, Seeman and Lee (15) determined that the half-maximal, receptor-mediated inhibition of the electrically stimulated release of H-3 dopamine from the striatum in the intact rat, as measured in vitro using slices of striatum, is achieved at concentrations (IC 50%) of 95 n*M* and 12 n*M* for haloperidol and spiroperidol, respectively. An important advantage of such an in vitro model is that the drug concentration at the pharmacologic site of action can be determined directly. The tenfold concentration difference (95 n*M* compared with 12 n*M*) presumably reflects the tenfold greater receptor-binding affinity of spiroperidol [K_D = 0.32 n*M* (6)] relative to haloperidol [K_D = 3.3 n*M* (6)]. Therefore, to yield equal fractional receptor occupancies, and thus equal pharmacologic effects (in the preceding example, inhibition of electrically stimulated dopamine release), the striatal concentration of haloperidol must be ten times that of spiroperidol. Accordingly, on the basis of the receptor-blockade hypothesis of neuroleptic action (16), the average daily clinical dose of haloperidol would be ten times that of spiroperidol if their pharmacokinetics, bioavailability to brain, etc., were essentially identical. On this basis, a correlation between the dissociation constant K_D and the average daily clinical dose of neuroleptics [i.e., the correlation actually demonstrated by Creese et al. (14)], is expected. The average daily clinical dose, as determined by Creese et al. (14), of haloperidol (152 nmol/kg, range 34–340) is actually three times that of spiroperidol (58 nmol/kg, range 18–110); the qualitative agreement with the hypothetically expected tenfold dose difference illustrates the basis of the correlation demonstrated by Creese et al. (14). Using the striatal concentration-dose data for F-18 haloperidol (Fig. 2 of Ref. 10) and for H-3 spiroperidol (Fig. 3 of Ref. 17) (i.e., invoking the implicit assumption of Eckelman and Gibson) at the average daily clinical doses (14) of 152 nmol/kg (57 μg/kg) and 58 nmol/kg (23 μg/kg) for haloperidol and spiroperidol, respectively, we can determine that the dose-normalized striatal concentration for haloperidol [expressed as relative concentration (18), for example] is approximately five times that for spiroperidol. Thus, the therapeutically effective striatal concentration of haloperidol is 15 times that of spiroperidol, in qualitative agreement with the expected tenfold striatal concentration difference and the correlation demonstrated by Creese et al. (14). Note that even this relatively crude consideration of pharmacokinetic and bioavailability (yielding an observed fifteenfold compared with the expected tenfold difference in striatal concentration) seems to refine the correlation analysis of Creese et al. (14) (yielding an observed threefold compared with the expected tenfold difference in average daily clinical dose).

In closing, we take this opportunity to provide an estimate of the percent dose in whole brain of haloperidol and of spiroperidol at tracer doses (1 μg/kg). The percent dose in whole brain can be estimated as the striatal relative concentration [30 for haloperidol (10) and 0.26 for spiroperidol (Zanzonico PB, Bigler RE, unpublished results)] times the striatal percent of body mass [0.056% for rodents (19)] plus the cerebellar relative concentration [eight for haloperidol (10) and 0.072 for spiroperidol (Zanzonico PB, Bigler RE, unpublished results)] times the remaining brain percent of body mass [0.94% for rodents (19)], assuming that the cerebellar relative concentration approximates the relative concentration in the remaining brain. On this basis, therefore, the percent dose in whole brain at tracer doses in rodents is 9.2% for haloperidol and 0.082% for spiroperidol.

PAT ZANZONICO
RODNEY E. BIGLER
BERNARD SCHMALL
Biophysics Laboratory
Memorial Sloan-Kettering Cancer Center
New York, New York

REFERENCES

1. FROST JJ, KUHAR MJ: In vitro and in vivo labeling of neurotransmitter receptors. In *Radiopharmaceuticals: Structure-Activity Relationships*. Spencer RP, ed. New York, Grune & Stratton, Inc., 1981, pp 595–618
2. KAHN CR: Membrane receptors for hormones and neurotransmitters. *J Cell Biol* 70:261–286, 1976
3. COOPER JR, BLOOM RE, ROTH RH: *The Biochemical Basis of Neuropharmacology*. New York, Oxford University Press, Inc., 1978, pp 60–71
4. BURT DR: Criteria for receptor identification. In *Neurotransmitter Receptor Binding*. Yamamura HI, Enna SJ, Kuhar MJ, eds. New York, Raven Press Books, Ltd., 1978, pp 41–55
5. BURT DR, CREESE I, SNYDER SH: Properties of [^{3}H]-

haloperidol and [^{3}H]-dopamine binding associated with dopamine receptors in calf brain membranes. *Mol Pharmacol* 12:800-812, 1976

6. SEEMAN P: Brain dopamine receptors. *Pharmacol Rev* 32:229-313, 1980
7. LEFUR G, GUILLOX F, UZAN A: *In vivo* blockade of dopaminergic receptors from different rat brain regions by classical and atypical neuroleptics. *Biochem Pharmacol* 29:267-270, 1980
8. TEWSON TJ, RAICHLE ME, WELCH MJ: Preliminary studies with [^{18}F]-haloperidol: A radioligand for *in vivo* studies of dopamine receptors. *Brain Res* 192:291-295, 1980
9. BITTIGER H, BICHOFF S: *In vivo* [^{3}H]-spiroperidol binding: Characterization of dopamine and serotonin receptors in different areas of the rat central nervous system. In *Neurotransmitters and Their Receptors*. Littauer UZ, Dudai Y, Silman I, Teichberg VI, Vogel, Z, eds. New York, John Wiley & Sons, 1980, pp 67-71
10. ZANZONICO PB, BIGLER RE, SCHMALL B: Neuroleptic binding sites: Specific labeling in mice with [^{18}F]haloperidol, a potential tracer for positron emission tomography. *J Nucl Med* 24:408-416, 1983
11. ARNETT CD, FOWLER JS, WOLF AP, et al: Specific binding of [^{11}C]-spiroperidol in rat brain *in vivo*. *J Neurochem* 40: 455-459, 1983
12. LADURON PN, JANSSEN PFM, LEYSEN JE: Characterization of specific *in vivo* binding of neuroleptic drugs in rat brain. *Life Sci* 23:581-586, 1978
13. WAGNER HN, JR., BURNS HD, DANNALS RF, et al: Imaging dopamine receptors in human brain by positron tomography. *Science* 221:1264-1266, 1983
14. CREESE I, BURT DR, SNYDER SH: Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192:481-483, 1976
15. SEEMAN P, LEE T: Neuroleptic drugs: Direct correlation between clinical potency and presynaptic action on dopamine neurons. In *Antipsychotic Drugs: Pharmacodynamics and Pharmacokinetics*. Sedvall G, Uvnas B, Zotterman Y, eds. New York, Pergamon Press, 1976, pp 183-191
16. SEEMAN P: Anti-schizophrenic drugs—membrane receptor sites of action. *Biochem Pharmacol* 26:1741-1748, 1977
17. KUHAR MJ, MURRIN LC, MALOUF AT, et al: Dopamine receptor binding *in vivo:* The feasibility of autoradiographic studies. *Life Sci* 22:203-210, 1977
18. WOODARD HQ, BIGLER RE, FREED B, et al: Expression of tissue isotope distribution. *J Nucl Med* 16:958-959, 1975
19. GLOWINSKI J, IVERSEN LL: Regional studies of catecholamines in the rat brain-I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] DOPA in various regions of the brain. *J Neurochem* 13:655-669, 1966

Re: Thyroid Iodine Content Measured by X-Ray Fluorescence in Amiodarone-Induced Thyrotoxicosis

I feel compelled to comment on the article of Leger et al. (*1*) concerning the x-ray fluorescent (XRF) measurement of thyroid iodine stores (ITI) in hyperthyroidism, because they referred to our own work (*2*), in my opinion out of context. They quoted us to support their findings that most of their patients with Graves' disease (GD) presented with a normal ITI, whereas we specifically stressed the fact that in our experience about two thirds of these patients presented with an ITI lower than normal. Furthermore, in one of their own publications (*3*), the same group shows that 20% of their hyperthyroid patients had a subnormal ITI. A wide range of ITI values has been reported in the literature in normal glands as well as in thyroid disease, the trend being that higher values are found in areas where iodine is supplemented in the diet. It has therefore been suggested that XRF measurements might be less useful from a clinical and individual point of view in these areas (e.g., the U.S.), than in other parts of the world (*4*).

As far as the main subject of the article is concerned—namely ITI in amiodarone-treated patients—our own experience is also at variance with the author's data. We have examined many patients taking amiodarone and, although only one short series has been formally published (*5*), we consistently find that the patients under amiodarone therapy that remain euthyroid generally accumulate a significant amount of iodine in their glands. The nature of the mechanism for this remains unclear (*6*); there is a wide range (20 to 100 mg) and a mean of about four times normal (35 mg as opposed to 9 mg). Most of the patients becoming hyperthyroid while on amiodarone therapy, but not all, fall in this range, so that an ITI determination is not diagnostic in this situation. On the other hand, we agree that the ITI evolves in close parallel with the thyroid state in treated patients or in patients recovering or recurring spontaneously (*5*), making the XRF measurement a very useful tool for follow-up. We feel that it is at this place that the authors should have referred to our article (*2*) because hyperthyroid patients taking amiodarone behave like the hyperthyroid patients with iodine overload that we described, as far as the relationship between ITI and circulating hormones is concerned. It is also our experience that when the amiodarone-treated patients have antibodies against the thyroid, they have a subnormal ITI and are either hyperthyroid (GD) or hypothyroid. We therefore use the information yielded by means of XRF in a way different from that proposed by the authors: if a patient taking amiodarone presents with an ITI lower than 20 mg, and has been on the drug for a period longer than 3 mo (it takes about 6 wk to obtain a plateau of the ITI), he is strongly suspected of developing either hypo- or hyperthyroidism and is very closely examined and followed up.

M. H. JONCKHEER
Academic Hospital V.U.B.
Brussels, Belgium

REFERENCES

1. LEGER AF, FRAGU P, ROUGIER P, et al: Thyroid iodine content measured by x-ray fluorescence in amiodarone-induced thyrotoxicosis: Concise communication. *J Nucl Med* 24: 582-585, 1983
2. JONCKHEER MH, DECONINCK F, SWAENEPOEL L: Upon the importance of differentiating between two forms of hyperthyroidism by means of x-ray fluorescence scanning. In *Thyroid Research VII*. Stockigt JR, Nagataki S, eds. Australian Academy of Sciences, Canberra, Austr. 1980, pp 637-640
3. FRAGU P, SCHLUMBERGER M, AUBERT B, et al: Thyroid iodine content measurement helps for the diagnosis of hyperthyroidism with undetectable radionuclide uptake. In *X-ray Fluorescent Scanning of the Thyroid*. Jonckheer MH, Deconinck F, eds. Boston, Martinus Nijhoff Publishers, 1983, pp 145-162
4. JONCKHEER MH, WAHNER HW: Clinical usefulness of X-ray fluorescence thyroid iodine quantitation and scanning. In *X- ray Fluorescent Scanning of the Thyroid*. Jonckheer MH, Deconinck F, eds. Martinus Nijhoff Publishers, Boston, 1983, pp 163-180
5. JONCKHEER MH, HUYGHENS L: Effects of amiodarone on the thyroid gland. In *New Aspects of the Medical Treatment Of Tachyarrhythmias*. Breithardt G, Loogen F, eds. Munchen